DETERMINATION OF DISSOLVED ORGANIC COMPOUNDS IN NATURAL WATERS

BJÖRN JOSEFSSON

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DEPARTMENT
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GÖTEBORGS UNIVERSITET — CHALMERS TEKNISKA HÖGSKOLA

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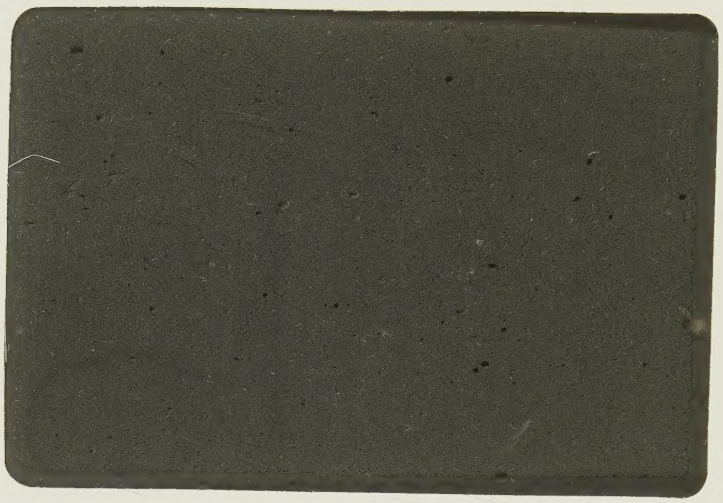

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PREFACE

This article summarizes the field in which the author is active and constitutes the formal thesis for the doctorate in chemistry at the University of Gothenburg. The thesis also consist of the following papers (referred in text with roman numbers I - V):

I Determination of soluble carbohydrates in sea water by partition chromatography after desalting by ion-exchange membrane electrodialysis. B.O. Josefsson, Anal. Chim. Acta, 52(1970)65.

II Automatic spectrophotometric procedures for the determination of the total amount of dissolved carbohydrates in sea water. B.O. Josefsson, L. Uppström and G. Östling, Deep Sea Res., 19(1972)385.

III A fluorimetric determination of lignin sulfonates from natural waters in the presence of humic substances. T. Almgren and B.O. Josefsson, Sv. Papperstidning, 76(1973)19.

IV A simple apparatus for on site continuous liquid-liquid extraction of organic compounds from natural waters. M. Ahnoff and B.O. Josefsson, in press, Anal. Chem.

V Confirmation studies on polychlorinated biphenyls (PCB) from river waters using mass fragmentography. M. Ahnoff and B.O. Josefsson, Analytical Letters, 6, No 12(1973).

The author wishes to thank Professor David Dyrssen for valuable discussions and support during the work. I am also indebted to my co-workers Dr Leif Uppström, Mr Martin Ahnoff, fil.mag., Mr Tom Almgren, fil.kand. and Göran Östling, fil.kand.. Many thanks are also due to Mrs Ulla Holmberg for typing the manuscripts, to Mr Kenneth Berndtson for photographing the figures and Olof Hansson for preparing the mimeographs. I should like to thank Dr Linnéa Lillienberg and my colleagues at the Department of Analytical Chemistry, University of Gothenburg, for pleasant cooperation and Dr Enrica Ratti-Moberg for revising the English text. This work was supported by grants from the Swedish Natural Science Research Council, Knut and Alice Wallenberg's Foundation and National Swedish Environment Protection Board.

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BACKGROUND

Dissolved organic compounds in natural water.

Dissolved organic compounds in the sea are introduced from several sources. The largest fraction probably originate from organisms especially phyto- and zooplankton. River drainage and atmospheric fallout may also contribute with organic matter from terrestrial sources. Marine pollution plays an ever increasing role, especially those compounds which are foreign to marine organisms. The amount of dissolved organic material in the sea, estimated by Parsons[1] ten years ago, is considerably greater than the amount of particulate matter (see Fig. 1).

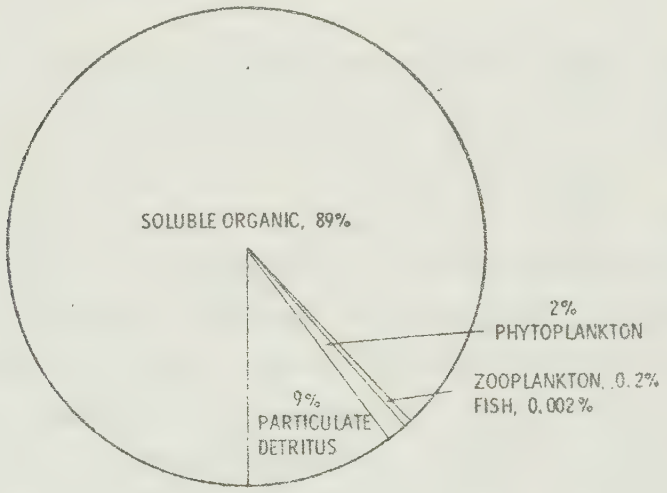

Figure 1.

However, the distinction between the two categories is still a problem since it is dependent on the filtering and centrifuging procedures. Whether the dissolved organic material in natural water is in true solution or in the colloidal state is unknown. Because of the very low concentrations involved it will be extremely difficult to solve this question.

The ease by which organic matter is absorbed on iron and aluminium hydroxides suggests that in the sea some of the organics are absorbed on colloidal and larger particles. Postma and Kalle[2] have shown that high concentrations of colloidal organic matter discharged into the sea by rivers coagulated within a short distance off the coast. However, the coagulation process is less likely in open sea because of the extreme dilution. Dissolved organic matter is generally considered to be that which passes through a 0.45 µm Millipore HA membrane filter or Selas Flowtronics silver filters of varying porosities. Thus the dissolved organic matter includes colloidal as well as truly dissolved meterial.

The concentration of dissolved organic matter in sea water ranges from 1 to 5 mg C/l, but in polluted areas it can be several times more. In limnic waters there is a broader range of concentrations.

The biochemical effect of the dissolved organic substances.

An important aspect of the study of dissolved organic constituents of natural waters is the understanding of the ecology or flow of organic matter in the water. Lucas[3] suggested the term ectocrines for ecologically potent metabolites extruded into the water. These external metabolites, of which very little is known of their source and nature, are responsible for the variations in the biological character of the water. The photosynthetic organisms were thought to be correlated with mineral nutrients such as nitrogen, phosphorous and trace metals, which are present in waters in limited quantities. However, this was found to be insufficient in many waters, and Provasoli[4] showed that many algae need vitamins, which they could not produce themselves. It is now being realized that ectocrines from some water organisms may effect the growth of other organisms by producing necessary nutrients, (e.g. vitamins), by removing inhibitory compounds (e.g. masking), by producing toxic or repellant substances (e.g. antibiotics).

The olfactory sense is highly developed in many marine organisms and probably serves several useful functions. Von Frisch[5] showed that the recognition and location as well as alarming of other individuals are controlled by chemosensors. Hasler[6] reported that young fish can be conditioned to the types of water in which they have developed so that they are later attracted to them. Thus, mature fish can and do differentiate between waters of their native and other streams. This differentiation is mediated by the organic waste substances in the stream, which presumably differ from stream to stream.

In recent years man-made organic compounds have been produced for various purposes, e.g. chlorinated compounds and pesticides, and many of these resistant compounds are accumulated in the sea. Organic chlorinated compounds such as PCB and DDT have already become surprisingly widespread througout the oceans. There are subtle aspects in water quality that are recognized as impacts such as taste and odor of water, chronic toxicity to aquatic life or the concentrating of hazardous materials up the food chains.

Analysis of small quantities in complex mixtures.

In recent years fast development of analytical techniques and adequate sampling apparatus has increased the possibilities to approach the determination of minute constituents in complex mixtures. The knowledge of the dissolved organic compounds in natural waters is a direct result from the application of sophisticated instrumentation. Riley[7] stated that the major developments in the sea water chemistry will probably centre around the dissolved organic compounds and their biochemistry.

Because natural waters may be very different, the analytical methods must be adapted to the type of water. Sea water causes special complications because the salt content is about 10000 times greater than the dissolved organic matter.

Methods used for sea water analysis can often be adapted to limnic waters, but the reverse is not necessarily true. Body fluids from human beings have an ionic strength which is only somewhat smaller than that of sea water, but the order of magnitude of dissolved organic constituents is much higher.

Natural waters contain mixture of a tremendous number of compounds. There are well known metabolites released from cells mixed with unknown substances of biological origin. In addition man-made synthetic compounds will increase the difficulties of characterizing the mixtures. The number of compounds which are abundant in a water sample are limited, but there is an overhelming number of substances at the lowest levels of concentration where only a few molecules are present. The analytical task is to reach as low a level as possible in order to identify important biologically working chemical sensors. As a practical example, a dog will recognize butyric acid in the air by its odor in concentrations as low as approximately 10^{-3} ppt. He will need a minimum quantity of about 10^{-16} g of butyric acid. In water the eel has been found to react to a β-phenylethylethanol concentration of 1800 molecules per ml of water[8]. In comparison the most sensitive detection so far with a gas chromatographic electron capture detector is 4×10^{-13} g of beryllium as volatile beryllium(II)trifluoroacetylacetonate[9].

General problems in trace analysis.

Trace organic analysis has not yet reached its theoretical limits. The problem seems to be that the systematic error often exceeds the random error by several orders of magnitude. Working with extremely low concentrations in the sample requires very careful handling and the use of combination of many enrichment and separation processes all of which have to be checked. During the processes the trace substance can react and decompose, thereby the results

are systematically falsified. The statistical random error can be detected easily by repetetive sampling, analysis and evaluation, but it is difficult to find the systematic error.

In water analysis these systematic error can be introduced in all steps:

1. Sample collection.
   Environmental influence, e.g. oxygen, light, temperature and biological activity.
   Contamination from boats, pumps, tubes, adsorption on walls during storage and so on.
2. Enrichment and separation.
   Chemical reactions, incomplete separation, interphase adsorption and biodegration.
3. Detection and evaluation.
   Non-linearity in the range, detection artefacts, interferences, contaminations and inadequate calibration.

METHODS

Introduction.

The choice of parameters for analysis depends on the type of information sought. There is little possibility of applying a quantitative method which covers a wide spectrum of specific organic compounds of different classes. When selecting an analytical method the analyst should carefully distinguish between specific and nonspecific methods. Nonspecific methods are connected to organics with typical functional groups, i.e. sugars or aminoacids. Nonspecific methods to determine the total organic content of the water is not discussed here. The selection of an appropriate method depends on the character of the organics as well as the amount present. Substances, previously not described or found in natural waters, will give rise to great analytical problems from the enriching step to the structural determination. In such cases a specific biological assay is very important in combination with chemical analysis.

The direct analysis of aqueous solutions is in most cases impossible. However, some improvements have been made in this field in the last few years (see A below).

Generally the characterization of the organic trace substances has been undertaken by combining a concentration procedure with an analytical technique (see B below). The concentration of the sample serves to reach the detection limit of the analytical apparatus and to separate the constituents of interest from interfering species.

A. Direct methods. These methods are applied directly to the water sample with no complicated concentration or separation steps. The advantage is a simple sampling procedure giving a good representative sample. However, the specificity is rather poor except for enzymatic methods.

Most common procedures use spectrophotometric detection in combination with a specific color reaction. The possibility of automating the procedure is shown in paper II for dissolved carbohydrates in sea water. Since the manual carbohydrate determinations are rather difficult to reproduce, the advantage of the automatic system is obvious. Its processing speed is 240 determinations an hour. However, the color reactions are not strictly specific for carbohydrates. The methods are attractive, while the great amount of information is suitable for studies of e.g. photosynthetic activities in different waters. Absorbtiometric measurements made with a spectrophotometer or colorimeter can at best detect colored materials in concentrations of 0.1 ppm.

Fluorescence techniques are becoming more common and more widely applied in water analysis. Generally, in a series of aromatic structures, those which are the most rigid, planar and sterically uncrowded will tend to be the most fluorescent. The increased interest in these compounds is due to the fact that they are extremely potent for biological activity, e.g. vitamins, hormons, toxins and cancerogenes.

Fluorescence analysis has several advantages over absorption spectrophotometry which make it to an extremely powerful analytical tool. First, the intensity of fluorescence is directly proportional to the concentration of fluorescent material and generally linear in a concentration range of about 10^4. Secondly, the sensitivity is extremely high and the sample must be sufficiently dilute so as not to attenuate the exciting beam of light by more than about 5 % at most, otherwise the sample absorption causes inner-filter effects. The high sensitivity is due to the fact that the multiplier photocell sees the emitted light against a dark background, whereas in observing the absorbance of the same solution, it is necessary to measure the removal of a very small fraction of the radiation. This leads to the difficulty of measuring the difference between two large numbers. Fluorescence methods offer additional instrumental parameters which can be varied independently to increase specificity.

In paper III a fluorimetric method is used to study lignin sulfonates in natural waters. Water soluble "lignins" released by digestion of wood with aqueous bisulfite (Sulfite process) or aqueous alkali (Kraft process) are polymers containing different amounts of sulfur depending on the cooking procedure. These aromatic phenolic type compounds of complex structure are known to be highly toxic to various marine organisms[10]. Because of the polyaromatic structure of the water soluble "lignins" they are strongly fluorescent and can be determined directly by fluorimetry without the addition of dyes or reagents. The method of constant addition used in paper III is of particular use for samples in complex matrices where background emission of humic substances may influence the emission of the sample.

Enzymatic assays are useful because of their high specifity, but they are limited by the instrumentation. Hicks and Carey[11] increased the sensitivity of an enzymatic assay for glucose by a fluorescence technique, and used it for the direct determination of glucose in natural waters at the lever at 10^{-8}M

without prior concentration. Hexokinase reacts quantitatively with glucose to give glucose-6-phosphate which is allowed to react with enzyme glucose-6-phosphate dehydrogenase to produce the reduced form of NADP. The NADPH reduces the dye resazurin to a highly fluorescent product resarufin. The amount of resarufin is proportional to the glucose concentration in the original sample.

B. Indirect methods.

General methods for concentration and isolation of organic substances.

The concentrations of the numerous dissolved organic compounds of fresh water and sea water are so low that it is almost always necessary to concentrate and separate them from the inorganic salt matrix prior to analysis. A thorough knowledge of the limitations inherent in each procedure is necessary in order to select the most suitable method. The attainable range of concentration of organics will vary significantly with the final accessible instrumental methods and the concentration must be accomplished without decomposition, alteration or contamination.

Jeffrey and Hood[12] have investigated the efficiency of several methods to concentrate small amounts of biologically important compounds, some labelled with ^{14}C, dissolved in sea water. They also employed a ^{14}C labelled aged algae culture as the test solution to evaluate the various isolation and separation methods. A review of concentration and separation techniques for the analysis of organic compounds has recently been written by Andelman and Caruso[13]. At our laboratory we have extensively studied different concentration techniques. Some experiences will be reported here, but details are found in papers I, III and IV.

Activated carbon filters. This is the most widely used procedure to concentrate all kinds of organics from fresh water and sea water. However, the method has a number of shortcomings.

The adsorptive capacities will as well as desorption feasibility vary considerably. Alteration of the organics occurs on the carbon surface by oxidation. Biological degradation is difficult to control. Analysis of volalite odorous compounds from river water is troublesome because when the filter is dried at room temperature, before the extraction with organic solvents, the trace substances will codistillate with the water. An advantage is that one can pump large amounts of water through the filter and apply it in situ. Carbon filters seem to be appropriate in waste water analysis.

Ion-Exchange. To use ion-exchange resins for desalting sea water prior organic trace analysis is practically impossible. The quantity of resin needed to desalt one liter of sea water (35 °/oo salinity) will release too much contaminating organics in comparison with the naturally organic matter. However, enriching humic acids from fresh water has been reported[14]. Reactions on ion-exchange resins in their acid and base forms may occur and often leads to undesirable products. Sugars e.g. epimerizes on an ion exchange resin in the OH^- form.

Ligand-Exchange Chromatography. Ligand-exchange was first described by Helfferich[15]. The chelating resin is a styrene--divinylbenzene copolymer to which e.g. iminodiacetic acid functional groups (half EDTA) are attached. This resin is extremely selective for heavy metal ions, which are more strongly bound to the chelating resin than to monofuntional cation exchangers. Consequently there is little tendency for copper or iron to be displaced from the resin. The ion--exchanger is then used a a solid carrier for the complexing metal ion which in turn can form complexes with ligands which may be anions or neutral molecules such as ammonia, amino acids or humic substances. When, e.g., sea water is passed through a ligand-exchange column the ligands are preferentially sorbed, while nonligands and the salt pass through.

The ligand can later be recovered from the column by displacement with another more strongly complexing ligand. The advantage of this type of ligand-exchange is that complex formation is, as a rule, a more specific interaction than ordinary physical sorption or ion-exchange.

This technique was first used by Siegel and Degens[16] to concentrate dissolved amino acids from sea water. They used Chelex-100 resin in the copper form and eluted the amino acids with ammonia which was easy to evaporate.

Humic substances are very complex and contain aromatic groups, sulfur, nitrogen and oxygen. These substances are known to form complex with iron. An iron(III) ligand-exchange column was prepared to enrich humic substances from river water. In this way water-soluble humic substances were isolated. In paper III these substances were examined for their fluorescence properties.

Ion Retardation. A very special resin is Retardion 11 A8 which can retard dissolved salts, especially monovalent ions, and allowing non-electrolytes to emerge from the column first. In this way neutral and large molecules can be separated from salt. The resin is an amphoteric polystyrene, polyacrylate ion-exchanger which has negativ cha ges trapped inside a network polymer with charges of opposite sign. The resin swells in water by hydration and absorbs salts much more strongly than non-electrolytes. The resin was tried for the separation of sugars from salt in sea water but it was difficult to remove the sulfates even when a series of columns were used (see paper I). It is possible that this method can be used in fresh water, but no results are reported.

Electrodialysis with ion-exchange membranes. Electrodialysis is a process in which electrolytes pass through membranes as a result of the application of an electric field. Although it resembles ordinary dialysis in its dependence upon membranes and in providing compartments and passages for the flow of solutions, the two processes are

distinguished from one another by differences in equipment design, objectives and membrane technology. In electrodialysis it is possible to drive electrolytes from a dilute to a more concentrated solution therefore it may be used for the removal of the sea salt.

The principle is old and it was first practiced by Schwein[17] in 1901 for purifying sugar extracts. Pauli[18] in 1913 was the first to apply a three compartment electrodialyzer as shown in Fig. 2.

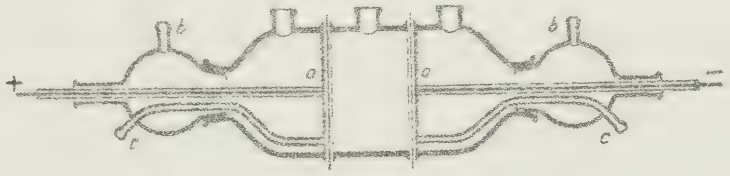

Figure 2.

The platinum gauze electrodes were placed next to the membranes to reduce the polarization at the membrane surface to a minimum.

Modern applications of electrodialysis are dependent on the development in recent years of membranes which are selectively permeable to anions or cations. The plastic cation membrane operating under a d.c. potential, allows positively charged ions such as sodium to pass through. Fixed negative groups distributed evenly within the insoluble matrix of the membrane prevent passage of anions through the membrane. The anion membrane is constructed similarly and operates in like fashion, but is selective for anions. It is a great advantage to desalt sea water in the determination of residual sugars (see paper I) since they are neutral and therefore not influenced by the electrical field. The desalting procedure can be carried out under sterile conditions so that the sugars are not degraded by microorganisms.

The speed of desalting depends on the current density. A current density of 25-30 mA/cm^2 can be used with the Permaplex membrane. In an apparatus (similar to that one in paper I), designed for a volume of 2 liters, the sea water was desalted in 8 hours by applying 3 A at the beginning (15 mA/cm^2). When the sea salt is exhausted in the sample the current drops to almost zero.

Liquid-liquid extraction. There are two ways of extending the limit of detection of trace substances in water. First, is to use the highest instrumental sensitivity and second to concentrate the materials to be analyzed from as much water as possible. When the instrumental detection limit is attained one must then optimize the concentration step. One possibility is to use continuous liquid-liquid extraction. In paper IV a new construction of a continuous liquid-liquid extraction apparatus is described which fulfills this purpose. Several extractors have appeared in literature that separate and concentrate a great variety of organic materials from water either by serial or continuous liquid-liquid extraction. Simple serial batch extraction to prepare organic concentrates from natural waters is an attractive method for moderate concentrations, since little time is required. With solvent extraction the sample is more likely to maintain its original form from the time of collection to the final analysis. By solvent extraction with suitable solvents the organic substances are withdrawn from the aqueous phase thus preventing biological or physical degradation. The procedure avoids exposure of the extracts to heat or solid surfaces which can affect labile organics. The solubility characteristics of dissolved organic substances are so diverse that any single solvent can, in principle, recover only a small fraction of the total organics under the best circumstances. The liquid-liquid extraction process can be compared with solvent extraction of the dried activated carbon in the carbon adsorbtion procedure. No one solvent would remove all the organics, but selected classes of organics could be removed by the proper choice of solvent.

Theory. The partition coefficient is given by the ratio of activities of a solute in the two phases (organic immisible solvent and water) and is called the Nernst partition law.

$$P = \frac{X_{org}}{X_{aq}} \qquad (1)$$

Normally the ratio of concentrations may be used because the extraction involves molecular rather than ionic species and dilute solutions. Polymerization will not be considered. Thus in practical application the distribution ratio will be defined by the distribution constant

$$K = \frac{C_{org}}{C_{aq}} \qquad (2)$$

The fraction of material originally present in the water and extracted by the organic solvent is

$$F_{eq} = \frac{1}{1 + \frac{V_{aq}}{K \cdot V_{org}}} \qquad (3)$$

where F_{eq} is the fraction extracted and V_{aq} and V_{org} are the volumes of the aqueous phase and of the organic phase. This relationship is useful in determing the number of extractions required to quantitatively enrich a specific organic compounds from water. The extraction efficiency is dependent on the distribution constant and the phase volumes. A series of extractions from water using a fresh solvent is the usual procedure since one extraction with a given volume of solvent is less efficient than two extractions using half of the solvent each time.

Another consideration is the concentration factor, the ratio between the concentration of extracted material in the organic phase at equilibrium compared to the initial concentration in the water.

$$\frac{C_{org}}{C_{aq,init}} = \frac{1}{\frac{1}{K} + \frac{V_{org}}{V_{aq}}} \qquad (4)$$

It is thus important that the distribution constant will be large because of the small amounts to be extracted from a large volume of water. The smaller the volume of the extracting agent used, the greater will be the concentration factor, but then the recovery will be lower.

Practical applications of liquid-liquid extraction.

Batch extraction. Serial batch extractions are the most common methods because of their simple and fast performance. Because of the small concentrations of organics in sea water Cronin[19] extracted the water in three specially constructed 200 liter drums. He manually mixed 151 liters of water three times with 19 liters of petroleum ether. Totally 2000 liters of water were extracted with the same solvent. Veith[20] determined PCB in 20 liters of river water by shaking 1.6 liter portions with 400 ml of hexane. The process was repeated until the 20-liter sample was extracted twice and then the hexane portions were combined.

Semi-automatic methods. A semi-automatic procedure was introduced by Kawahara et al.[21] using a magnetic impeller placed in the cap of an inverted sampling bottle to create vortex mixing. One liter samples were extracted with 50 ml of solvent achieving 50 % more efficient recovery than by the manual procedure. Schaffer et al.[22] used a similar method extracting 3.5 liter samples twice with only 10 ml of hexane and giving a concentration factor over one hundred.

Counter current methods. Counter current extraction procedures have been developed by Craig and Scheibel. The commercial extraction apparatus by Scheibel consists of ten mixing stages and in between ten settling stages. The extracting lighter than water solvent is passed in a counter current to the water and is recycled to reduce the final solvent volume. Hoak[23] showed that the extraction efficiency of the Scheibel apparatus was directly proportional to the agitator speed for a given feed rate of water. Hoak[23] found that problems arose when the river water tended to form an emulsion with the solvent. Cronin[19] used a one meter pulse column on board a ship to extract orga-

nics from sea water with methyl isobutyl ketone. He got a solvent recovery between 26.5 and 77 percent depending on volume of solvent used and pH of the water. Bunch and Ettinger[24] constructed a field counter current liquid-liquid extractor with the Podbielniak model 6000 SP centrifugal contractor incorporated. This extractor give multiple-stage extraction with a minimum amount of solvent. Besides it could also operate on both heavier and lighter than water solvents. The ratio of solvent to water was 1:10 to 1:100. Two trucks were used to carry a completely portable unit for field applications.

Continuous liquid-liquid extractors. A small liquid-liquid extractor for laboratory use was designed by Kahn and Wayman[25]. This apparatus was used with multiple mixing chambers and internal solvent recycling during continuous water flow. Goldberg[26] modified it for heavier than water extraction. Later Goldberg[27] simplified the apparatus leasing out the magnetic stirring system. Instead the recycled solvent was passed through a porous glass frit. The frit homogenizes the solvent resulting in fine drops which rise through the incoming water in the column. The solvent recycling system in these laboratory extractors works by evaporating the solvent in a flask and passing it through a condenser at the top of the equipment. From the condenser the solvent flows through a funnel tube to the bottom of the aqueous stream where it is dispersed. After passing through the water layer the extract flows into the evaporation flask where the solvent is redistilled. This system for recycling the organic solvent is also used in Scheibel apparatus.

Our own continuous liquid-liquid extraction apparatus is described in paper IV. It was developed from experience with most of the previously mentioned extraction procedures. Our intention was to construct an apparatus as simple as possible which would fulfill the requirement of efficiency and in situ operation. The field or in situ operation was of highest importance, since it is impossible to transport large volumes

of water without great alteration of the chemical micro constituents of the water. The apparatus must be of non--contaminating materials (teflon, glass). In addition, the extractor should be simple to handle and it should work automatically on the field. In addition large amounts of water should be processed with a minimum of organic solvent and with sufficient contact time. Redistillation is avoided since co-distillation may occur and because exposure to hot surfaces may cause decomposition.

As described in paper IV the extractor is a mixed settler without a solvent refreshing system. The organic lighter than water solvent is trapped inside the extractor. The limiting factors in the system are the solvent saturation capacity and the water solubility of the solvent in water. When studying low concentrations of organic compounds with very high distribution constants such as the highly lipophilic DDT and PCB the organic solvent will not be saturated. When the organic solvent is saturated the analytical detection limit is well exceeded. Because of the simple construction of the apparatus it was possible to arrange a series of extractor trains. The amount extracted in each individual extractor in a series could be used in the calculation of the amount present in the water.

For the development of quantitative liquid-liquid extraction techniques the choice of solvent is important. In addition water quality parameters must be considered. The proper choice of solvent depends on the following factors:

1. Solubility in water. The extracting solvent should be essentially immiscible with water and vice versa.

2. Character of the solvent such as polarity and aromaticity.

3. Volatility. Since the recovery of the solute low solvent volatility will usually simplify this procedure.

4. Purity. It is extremely important to use very pure solvents when isolating trace organic compounds by concentration procedures.

5. Viscosity and density. The solvent properties should be considered in order to minimize unwanted emulsions.

The following properties of water should be considered:

1. Ionic strength. In sea water (ionic strength 0.7 M) a salting-out effect occurs, which may increase the extractability of organics from the water. River waters have a considerably lower salt content (ionic strength about 0.01 M).

2. Particles. Occlusion and adsorption of organic molecules on particulate matter influences the liquid-liquid extraction process. The problem is not solved by filtering the water.

3. Coextraction of organic interferences. This problem is usually eliminated by a proper clean up step. Sulfur can be a severe problem in PCB and DDT analysis since it is soluble in nonpolar solvents.

4. The pH value. The pH can influence the stability of the organics as well as dissociative and associative properties.

5. Temperature. The preformance of the extractor is dependent on the water temperature. Cyclohexane, e.g., has a melting point of 6.55°C.

Other techniques.

Other techniques for concentrating organic compounds from natural waters will be mentioned briefly.

Freeze concentration. Shapiro[28] and Buker[29] have studied enrichment of organic matter by freezing out the water. The technique was applied to river and lake waters. The reduced temperature offers good protection of organics in the sample.

Coprecipitation. Enrichment of organic matter by coprecipitation has been applied to sea water. If the pH of sea water is adjusted to 10 a precipitate of magnesium hydroxid is formed containing much of the organic material. The same effect can also be achieved with iron(III) chloride which produces an iron(III) hydroxide floc which coprecipitates the organic material. Some of the organic material can be removed from the floc with e.g. chloroform.

Different adsorbtion filters. A lot of new adsorbents have recently been described[30,31]. The most promising seem to be the macroreticular ones manufactured by Rohm and Haas. Generally they are not as efficient as activated carbon for enriching organic material, but the organics are extracted more easily from the filter.

Some other techniques involved in concentration of organic compounds from water are distillation[32], molecular sieve filtration[33], dialysis, bubble separation[34] and foam fractionation[35].

Chromatographic separation

The mixutre of organic compounds, which is the usual result of the different concentration methods mentioned above, must generally be separated before the individual compounds can be determined. Fractionated extractions by solvents with different polarity and chromatography are the most common procedures. Numerous articles and books have been published in the field of chromatography. Only the separation techniques applied in the papers I, III, IV and V will be considered here.

The chromatographic procedures used separately or in combinations are:

liquid phase separation

liquid-liquid chromatography with ion exchange columns
liquid-solid chromatography with activated adsorbents
liquid-liquid chromatography with de-activated adsorbents

gas phase separation

gas-liquid chromatography.

Liquid-liquid chromatography with ion exchange columns

In paper I ion-exchange chromatography is applied to sugar analysis. The methods have been worked out by Samuelson and co-workers[36, 37]. High pressure chromatographic runs were made on either the cation exchange resin Dowex 50W-X8 with particle size 14-17 μm in the

lithium form or on the anion exchange resin T5C (Technicon) with particle size 10-17 μm in the sulfate form. The separations were carried out at 75°C and with 85 per cent ethanol as eluant. The cation exchanger in the lithium form gave the best separation of the disaccharides. The monosaccharides found in sea water were best resolved when the anion exchanger in the sulfate form was employed. The sugars in the eluate were automatically determined in a Technicon Auto-Analyzer with the orcinol sulphuric acid reagent. This reagent is very specific for carbohydrates since orcinol reacts with furfural and hydroxymethyl furfural which are formed on acid hydrolysis of sugars. Ethylene glycol and glycerol were separated on the cation exchangers in the lithium form and determined by periodate oxidation of the polyols to formaldehyde which gives a color reaction with pentane-2,4-dione.

From theoretical point of view, the separation process of sugars on ion exchangers in an ethanol-water media, is complex. The ratio water/ethanol is higher inside the resin than in the external solution. The polarity of the sugar as well as van der Waals forces result in retardation and separation by the resin. The type of ions inside the resin will also influence the distribution coefficients of the sugars.

Liquid-solid or adsorption chromatography

When natural waters are extracted with an unpolar solvent, many lipids and pigments are coextracted. These substances will seriously contaminate the determination of pesticides, especially when the electron capture detector is used. Liquid-solid chromatography techniques are used in so-called clean up fractionations. The activated adsorbent (Florisil, alumina or silicic acid) will efficiently retain the contaminants while the highly unpolar substances will be eluted with e.g. hexane. This technique is practized in PCB analysis reported in papers IV and V.

Liquid-liquid chromatography

In a study to differentiate PCB from other chlorinated compounds which would complicated subsequent qualitative and quantitative measurements a liquid-liquid chromatography system was investigated. The method was first reported by Reynolds[38] and based on the fact that the Florisil is partially deactivated by the addition of 3 per cent water which acts as a stationary phase. The chlorinated compounds were eluted with solvents of increasing polarity. However, the reproducibility and separation are affected by sample composition because the lipids effect the distribution between the solvent and the adsorbent. Furthermore, the separation is effected by the quality of Florisil and the aromatic content of the solvent[39]. Similar systems with silica gel have been reported[40], but all of them lack reproducibility.

Gas phase separation

Gas chromatography has an outstanding position in the analysis of trace organics because of the inherent advantages consisting of rapid analysis, good separation and high sensitivity. The object of the gas chromatographic analysis must be clear already in the concentration step of the organics from the water sample. The components must be volatile and the chromatographic parameters must be adapted for the desired separation and identification. Since many components of natural waters are completely unknown ancillary techniques are necessary to determine the sample composition.

Column performance sets the limit for the separation attainable. Grob[32] has recently reported a method to separate volatile substances in the surface water of Lake Zürich. He could by mass spectrometry identify 86 different organic compounds separated on a capillary glass column. However, many compounds of interest are not volatile. Therefore they have to be converted to stable derivatives suitable to gas phase analysis.

Volatile compounds from water. Chlorinated pesticides and PCB were determined by gas chromatography as described in papers IV and V. The PCB mixture was extracted from river waters together with other volatile compounds (mostly lipids). Before the gas chromatographic separation a

clean up procedure had to be performed.

The gas chromatographic behavior of PCB is very similar to that of some chlorinated pesticides so that this clean up must be made if the analytical results are to be reliable. The exact chemical composition of the PCB mixtures has not been determined. There are at least 70 different chlorinated biphenyls from theoretically 210 isomers, which are detectable in the 60 per cent chlorine technical standard[41].

The gas chromatographic pattern of the PCB residue in environmental samples have generally resembled commercial PCB with 54 and 60 per cent chlorine content, although the possibility exists that residues may be derived from any of the commercial products[42]. The gas chromatographic retention times, peak sizes, peak shapes and overall peak pattern must be carefully compared to the PCB standards used. Usually the PCB residue gives 14 peaks when using packed columns with non-polar silicone phases.

Nonvolatile compounds from water. By derivatization it is possible to convert very polar nonvolatile compounds to volatile and stable compounds which may be analyzed by gas chromatography. At the same time the derivatization can confirm the abundance of special classes of organic compounds. The active hydrogens

are replaced by different groups which reduce the polarity and decrease the hydrogen bonding. Hydroxy[43] and amino compounds,[44] regarded as nonvolatile or unstable at 200-300°C, have been analyzed in this way by gas chromatography after methylation, acetylation, trifluoroacetylation or silylation.

Since free sugars are unsufficiently volatile for direct gas chromatography their analysis requires the preparation of volatile derivatives. In paper I the sugars from sea water have been converted to TMS (trimethylsilyl) ethers. Since the sugars are isolated from water they have achieved mutarotation equilibrium. This means that up to 5 forms (α and β-pyranosides, α and β-furanosides and the linear form) can be silylated from one sugar. In practice 3 or 4 forms will appear in the gas chromatogram, but fructose may sometimes give 5 anomers. This multiplicity of peaks is impossible to resolve when many sugars are present as in sea water.

Detection and identification

The determination of the trace substances requires sensitive and selective detection systems. When a substance is first registered by a detector it is only an indication of its presence. The next step is to make use of all the information such as retention time, detector selectivity, method of concentration and clean up and type of derivatization. Often this information is not enough for a definite confirmation. Therefore special techniques must be applied such as mass spectrometry. Nuclear magnetic resonance and infra red are not discussed here since these techniques lack sensitivity and require relatively pure compounds. Detectors are characterized by the minimum detectable quantity that results in a signal, the concentration range to which the detector responds (dynamic range) and the concentration range over which the response is linear. Different detectors exhibit response patterns that are selective to particular organic compounds depending on the presence of particular elements or functional groups.

The electron capture detector is the most extensively used for chlorinated compounds such as PCB because it can detect as little as a few picogram of these compounds (see papers IV and V). The detector response differs for individual PCB mostly because of the number of chlorine atoms. This detector responds, however, to many other compounds such as metal organics, sulfur and phthalates.

The detector suffers also from a very limited linear range of response. In paper IV the PCB was indicated by matching retention times from three different columns against standards. This technique is not satisfactory for positive and absolute identification of the compounds extracted from aqueous environments. Since PCB is known to be very stable against strong agents, sulfuric acid (containing 10 per cent sulfur trioxide) was used to destroy as much as possible of the coextracted interfering materials. This clean up procedure increased the indication of the amount of PCB present. Berg[45] reported a method to quantitatively convert all PCB to decachlorobiphenyl with antimony pentachloride. In our case the total amount of PCB extracted was too small for derivatization with an acceptable yield. From the discussion above gas chromatography is a method to prove the absence of known compound, e.g. PCB, above a given concentration level. There is, however, a lack of positive structural information. To prove the presence of PCB at trace levels in a complex organic mixture, the most comprehensive technique for identification had to be used. This is the combination of a gas chromatograph with a mass spectrometer. The mass spectrometer will give the structural information needed. The electron capture detector can indicate at subnanogram amounts of

PCB. At this level ordinary mass spectrometer detection is not possible, Therefore a special technique was applied.

Mass fragmentography. By selecting the appropriate accelerating voltage when the magnetic field is constant the corresponding ion is focused. By quickly alternating the applied voltage corresponding to three preselected masses the different characteristic ions will be recorded simultaneously. Thus the mass spectrometer is used as a gas chromatographic detector with unique selectivity and at the same time a considerable gain in sensitivity is achieved. This technique was first applied by Sweeley[46] and later developed by Hammar[47], who called it mass fragmentography. The ratio of the intensities of the selected masses is characteristic for the compound. In paper V this technique was applied to detect and confirm the presence of PCB with four, five and six chlorine atoms. The naturally occurring stable isotopes of chlorine are ^{35}Cl and ^{37}Cl which are always abundant at a ratio of roughly 1:0.32. Thus the relative intensities for a compound with the following different number of chlorine atoms are:

number of chlorine	relative intensities
4 Cl	1:1.25:0.60
5 Cl	1:1.57:1
6 Cl	1:1.88:1.50

The mass spectrometer was focused on the parent peak and the two isotopic peaks, 2 units and 4 units higher, respectively. The electron beam was held at 20 eV to minimize fragmentation from higher chlorinated PCB which loose chlorines on ionization giving rise to fragment ions similar to the lower chlorinated ions focused.

Rote and Morris[48] have compared the theoretical intensities of PCB (taking also ^{13}C and ^{2}H into account) with the observed intensities. The following results were obtained:

PCB	molecular weight	m/e intensities theoretical	observed
tetrachlor	290, 292, 294	76:100:49	77:100:49
pentachlor	324, 326, 328	61:100:66	62:100:66
hexachlor	358, 360, 352	51:100:82	51:100:81

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Appendix I

Dissolved organic compounds in sea water

Carbohydrates

Aminoacids

Fattyacids

Other organic compounds

Carbohydrates

Compounds	Conc. range	Preconcentration	Method	Authors
Total carbohydrate	0-20 mg/l as arabinose in coastal water	none	Colorimetric with N-ethylcarbazole	Collier A. and Magnitzky W., Science III 151(1950)
Ascorbic acids and "rhammosides"	>100 mg/l coastal water	Adsorption on activated charcoal-celite, elution with ethanol	Spectrophotometry	Wangersky P., Science 115 685(1952)
Total carbohydrate	0.14-0.45 mg/l as sucrose in oceanic and coastal waters	none	Colorimetric with anthrone or N-ethyl carbazole	Lewis G.J. and Rakestraw N.W., J.Mar.Res. 14 253(1955)
Total carbohydrate	0.0-3.0 mg/l as arabinose in oceanic and coastal waters	none	Colorimetric with anthrone and N-ethyl carbazole	Collier A., Limnol. Oceanogr. 3 33(1958)
Galactose Glucose Mannose Rhamnose Arabinose	0.001-0.005 mg/l 0.005-0.015 " 0.003-0.017 " 0.001-0.007 " 0.0005 "	Extraction of dried acidified salts with 80% ethanol. The ethanol was evaporated. The residue was dissolved in water and desalted by ion exchange.	Paper chromatography	Degens E.T., Reuter J.H. and Shaw K.N.F., Geochim. Cosmochim.Acta 28 45(1964)
Total carbohydrate	0.16-0.45 mg/l as sucrose in oceanic waters	none	Colorimetric with anthrone	Srinivasagam T.R. Current Science 20 576(1965)

Carbohydrates cont.

Compounds	Conc.range	Preconcentration	Method	Authors
Total carbohydrate	0.40-3.17 mg/l as sucrose in coastal waters	none	Colorimetric with anthrone	Walsh G.E., Limnol. Oceanogr. 10 570(1965) 11 249(1966)
Glucose	0.002-0.078 mg/l oceanic and coastal waters	none	Enzymatic and fluorimetric	Vaccaro R.F., Hicks S.E., Jannasch H.W. and Carey F.G., Limnol. Oceanogr. 13 356(1968)
Total carbohydrate	0.2-1.2 mg/l as sucrose in coastal waters	none	Colorimetric with anthrone	Briggs R.B. and Wetzel C.D., Limnol. Oceanogr. 13 169(1968)
Total carbohydrate	0.1-0.5 mg/l	none	Colorimetric with phenol	Handa N., Organic Matter in Natural Waters, ed. D.W. Hood, University of Alaska 1970 p. 129
Ten monosaccharides and one disaccharide from which eight were quantified	0.15-46.5 µg/l coastal waters	Electrodialysis	Gas chromatography and ion-exchange chromatography	Josefsson B.O., Anal. Chim.Acta, 52 65(1970)
Saccharides	not known Black Sea water	Extraction of dried salt with acidified 80% ethanol. The alcoholic extracts were desalted by ion-exchange	Paper chromatography	Starikowa N.D. and Yablokova O.G., Okeanologiia, 12 431(1972)
Total carbohydrate	0.5-4.0 mg/l coastal and oceanic waters	none	Automatic colorimetric with five different reagents	Josefsson B., Uppström L. and Östling G., Deep Sea Res. 19 [illegible](1972)

Amino acids

Compounds	Conc. range	Preconcentration	Method	Authors
18 amino acids (including hydrolyzed)	3-130 µg/l oceanic	Co-precipitated with $Fe(OH)_3$, proteins hydrolyzed with HCl, Fe and cations separated by ion exchange	Paper and ion-exchange chromatography	Tatsumoto M., Williams W.T., Prescott J.M. and Hood D.W., J.Marine Res., 19 89(1961)
Total amino acids	2-100 mg/l coastal	none	Colorimetric with ninhydrin	Stephens G.C. and Schinske R.A., Limnol. Oceanogr. 6 175(1961)
Glycine threonine valine phenylalanine		Solvent extraction of 2,4-dinitro-phenyl amino acids	Circular thin-layer chromatography	Palmork K.H., Acta Chem.Scand. 17 1456(1963)
13 amino acids	0.5-37.6 µg/l oceanic	Extraction of dry acidified salts with 80% ethanol. Alcohol evaporated. Residue dissolved in water and desalted by ion-exchange	Paper chromatography	Degens E.T., Reuter H. and Shaw K.N.F. Geochim. Cosmochim. Acta 28 45(1964)
16 amino acids	0.5-29 µg/l coastal	Ligand exchange on copper-chelex 100 resin	Ion-exchange chromatography	Siegel A. and Degens E.T Science 151 1098(1966)
17 amino acids	0.5-20 µg/l eustarine	Ligand exchange on copper-chelex 100 resin. pH raised to 9.5 when concentrated.	Ion-exchange chromatography	Webb K.L. and Wood L. Automation in Analytical Chemistry. Technicon Symposium 1966 p.440

Amino acids cont.

Compounds	Conc.range	Preconcentration	Method	Authors
17 amino acids	0.55-16.85 µg/l eustarine	Ligand exchange on copper-chelex 100 resin. pH raised to 9.5 when concentrated	Ion-exchange chromatography	Hobbie J.E., Crawford C.C and Webb K.L. Science 153 1463(1968)
17 amino acids	1-25 µg/l coastal	Same as above, but pH was also adjusted to 7.0 to get the basic amino acids. The free fatty acids were extracted before the enrichment	Gas chromatography	Palmork K.H. International Council for the Eploration of the Sea. Hydrography Committee. CM 1969/C17
22 amino acids	not estimated	Dansylation and extraction with diethyl ether	Thin layer chromatography	Litchfield C.D. and Prescott J.M., Limnol. Oceanogr. 15 250(1970)
14 amino acids	0.5-134 µg/l coastal	Extraction of dry acidified salts with 80% ethanol. Alcohol evaporated. Residue dissolved in water and desalted by ion-exchange	Ion-exchange chromatography	Bohling H. Marine Biology 6 213(1970)
15 amino acids	0.2-8.1 µg/l coastal	Same as above	Thin layer chromatography	Riley J.P. and Seagar D.A. J.mar.biol.Ass.U.K., 50 713(1970)
12 amino acids	0.3-8 µg/l coastal	Same method as Webb and Wood	Ion-exchange chromatography	Andrews P. and Williams P.J. leB. J.mar.biol.Ass.U.K., 51 111(1971)

Amino acids cont.

Compounds	Conc.range	Preconcentration	Method	Authors
15 amino acids	0.1-15.6 µg/l oceanic	Freeze drying of the sample. Extraction of the amino acids from the salts with ethyl-ether and acidified ethanol. Salts of fatty acids were extracted with chloroform. Ion exchange clean up.	Gas chromatography	Pocklington R. Anal.Biochem. 45 403(1972)
18 amino acids	0.5-50 µg/l oceanic	Same method as Webb and Wood. In addition chloroform extraction of interfering contaminants followed by a final desalting step.	Thin layer chromatography	Clark M.E., Jackson G.A. and North W.J. Limnol.Oceanogr.,17 749(1972)

Fatty acids

Compounds	Conc.range	Preconcentration	Method	Authors
Acetic acid formic " lactic " glycollic acid	0.07-2.83 mg/l 0.03-1.03 " 0.02-0.13 " 0.06-1.40 " oceanic	Extraction at pH3 with chloroform or with ethyl ether	Partition chromatography	Koyama T. and Thompson T.G. AAAS Preprints of Int. Oceanogr.Congr. 1959 925.
Fatty acids: C_{10}-C_{22}	0.01-0.12 mg/l oceanic	Extraction with CS_2 or chloroform and Co-precipitation with $Fe(OH)_3$	Gas chromatography	Williams P.M. Nature 189 219(1961)
Fatty acids: C_{10}-C_{20}	0.1-0.8 mg/l oceanic and coastal	Extraction with ethyl acetate at pH2	Gas chromatography	Slowey J.F., Jeffrey L.M. and Wood D.W., Geochim. Cosmochim.Acta 25 607(1962)
Glycollic acid	0.1 mg/l coastal	Evaporation	Photometric with 2,7-dihydroxy naphthalene	Antia N.J., McAllister C.D. Parsons T.R., Stephens K. and Strickland J.D.K. Limnol.Oceanogr. 8 167(1963
Fatty acids: C_{10}-C_{22} 25 different saturated and unsaturated acids Besides were paraffinic hydrocarbons, sterols, triglycerides, fatty alcohols, vitamins, phospholipids etc. indicated	coastal and oceanic	Extraction with ethyl acetate and petroleum ether at pH2	Partition chromatography on silica gel column. The fractions were subjected to gas chromatography	Jeffery L.M., Pasby B.F., Stevenson B. and Hood D.W. Lipids of ocean water; in Advances of Organic Geochemistry eds. Colombo U. and Hobson G.D. Mac Millan Co., New York 1964

Fatty acids cont.

Compounds	Conc.range	Preconcentration	Method	Authors
Fatty acids: C_8-C_{22} Fatty esters. Fatty alcohols: dodecanol, cetyl alcohol. Hydrocarbons: pristane	Qualitative study of the oceanic surface films	Screen technique for sampling. Co-precipitation with $Fe(OH)_3$ and extraction with chloroform	Gas chromatography	Garrett W.D., Limnol.Oceanogr., 10 602(1965) Deep Sea Res., 14 221(1967)
Straight chain fatty acids (saturated and unsaturated) and singly branched fatty acids (iso- and anteiso acids): C_{12}-C_{19} Straight chain hydrocarbons: C_{14}-C_{33} Branched paraffins Isoprenoid hydrocarbons (pristane, phytane)	0.06-7.28 µg/l 0.0078-0.27 µg/l coastal	Extraction with n-pentane at pH2	Silica gel fractioning. Hydrogenation. Urea separation. Gas chromatography-mass spectrometry. Thin layer chromatography of unsaponifiables.	Blumer M., Organic Matter in Natural Waters, ed D.W. Hood, University of Alaska, 1970 p.153
Fatty acids: C_{12}-C_{18}	0.3-12.1 µg/l coastal	Extraction with n-pentane at pH2	Gas chromatography - mass spectrometry	Lillienberg L., Thesis. Dept. of Analytical Chemistry, University of Gothenburg 1971

Fatty acids cont.

Compounds	Conc. range	Preconcentration	Method	Authors
Total dissolved free fatty acid content	10-40 µg/l coastal	Chloroform extraction	Complexes of copper, fatty acids and triethanolamine are formed and quantitatively analyzed by atomic absorption spectrophotometry	Treuger P., LeCorre P. and Courtot P. J.mar.biol.Ass.U.K., 52 1045(1972)
		Other organic compounds.		
Aldehydes	0.01-0.04 mg/l (formaldehyde equivalents) oceanic	None	Photometric with 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) reagent	Kamata E., Bull. Chem Soc. Japan 39 1227 (1966)
n-Alkanes: C_{20} - C_{33}	0.2 µg/L (total amount) coastal	Extraction with n-heptane at lowered pH	Silica gel fractioning Gas chromatography	Peake E. and Hodgson G.W., J. Am. Oil Chem Soc. 43 215(1966)
Non aromatic hydrocarbons	Reltive to a standard. Baltic waters	Extraction with n-hexane	Liquid-adsorption chromatography on graphite column and heat of adsorption detector	Zsolnay A., Marine Chem. 1 127(1972)

Compounds	Conc. range	Preconcentration	Method	Authors
Hydrocarbons:	10-140 µg/L total in coastal. oceanic waters	Extraction with chloroform first twice pH 8 and then once at pH 2	The unsaponifiable fraction is cleaned up on thin layer plates and studied on gas chromatograph-mass spectrometer	Barbier M., Joly D., Saliot A. and Tourres D Deep Sea Res. 20 305 (1973)
Normal and branched paraffins	51.5%			
monocyclic naphthenes	5.5%			
bi -"- -"-	9.5%			
tri -"- -"-	6.5%			
monocyclic aromatics	18%			
bi -"- -"-	3.5%			
poly -"- -"-	2.5%			
Sterol, sterol esters	0.78-0.89 mg/1	Petroleum ether extraction at lowered pH	Thin layer chromatography	Adams D. and Richards A. Deep Sea Res. 15 471 (1968)
Choline type phospholipids		Ethyl acetate extraction at lowered pH		
Sulfhudryl compound (kind of mercaptan)	0.05-0.39 mg/1 (as mercaptans) anoxic fjord water			
Cholesterol	10 µg/1	Extraction with n-hexane at pH 2.5 -3.5	Thin layer and gas chromatography	Matthews S.W. and Smith L.L., Lipids 3 240(1967)
Stigmasterol	17 "			
β-sitosterol	135 " coastal			
Coprosterol (5β-cholestan-3β-ol) Cholesterol	Not given	Extraction with n-hexane	Thin layer in combination with gas chromatography	Smith L.L. and Gouron R.F Water Res. 3 141(1969)

Compounds	Conc. range	Preconcentration	Method	Authors
Methane ethane and ethylene propane propylene isobutane	$0.8\text{-}2830 \times 10^{-7}$ ml/l coastal and oceanic	Stripping off the gases with a purge gas(helium) and adsorbing the gases on activated alumina and activated charcoal	Gas chromatography	Swinnerton J.W. and Linnenbom V.J., Science 156 1119 (1967) J. Gas Chrom. 5 570 (1967)
Acetone buturaldehyde 2-butanone	5-53 µg/l 5-48 " 5-22 " oceanic and Mediterranean	Head space method	Gas chromatography	Corwin J.F., Bull. Mas. Res. 19 504(1969)
Urea	1-20 µg/l coastal	None	Photometric with biacetyl monooxim and semicarbazide reagents	Nevell B.S., Morgan B. and Cundy J., J. Mar. Res. 25 201(1967)
Urea ammonia	not given sea water	None	Photometric with hypochlorite and phenol	Emmet R.T., Anal. Chem. 41 1648(1969)
Urea	0.25-11.20 µg--atom urea N/l	None	Same as Nevell	Remsen C.C., Limnol. Oceanogr. 16 732(1971)
Creatine	0.05-0.22 µg--atom creatine N/l oceanic (Peru current)	None	Fluorimetric using alkaline ninhydrin Auto Analyzer	Whiteledge T.E. and Dugdale R.C., Limnol. Oceanogr. 17 309(1972)

Compounds	Conc. range	Preconcentration	Method	Authors
"Gelbstoff" and Humic substances	present coastal and oceanic	None	Fluorimetric	Kalle K., Oceanogr. Mar. Biol. Ann. Rev. 4 91(1966) Duursma E.K., Symposium "Optical aspects of Oceanography" Copenhagen 1972
"Organic matter"	present oceanic	None	Fluorimetric	Traganza E.D., Bull. Mar. Res. 19 897(1969)

Appendix II

Dissolved organic compounds in lake and river waters

Carbohydrates

Aminoacids

Fatty acids

Hydrocarbons

Humic substances

Other organic compounds

Carbohydrates

Compounds	Conc.range	Preconcentration	Method	Authors
Sucrose, glucose	ug	Desalting with ion exchange resins and evaporation	Paper chromatography	Vallentyne J.R. and Whittaker J.R., Science 124 1026(1957)
same as above besides amonioacids	same	same	same	Briggs M.H., Life Science 8 377(1962)
Total carbohydrate	0.2-1.0 mg/l	none	Colorimetric with anthrone	Sugawara K. and Kamata E. Nippon Kogaku Zasshi 86 1275(1965)
Glucose, fructose, galactose, sucrose	0.02-20 mg/l sewage samples	Evaporation	Gas chromatography	Bark L.S., Cooper R.L. and Wheatstone K.C. Water Res. 5 1161(1971)
Sucrose, raffinose, allulose, mannose, fructose, zylose or sorbose, glucose	ug/l levels municipal secondary effluent	The sample is reduced in the vacuum followed by freeze drying	High pressure anion exchange chromatography	Katz S., Wilson W., Scott C.D. and Rosen A.A. Water Res. 6 1029(1972)

Aminoacids

18 aminoacids	ug/l	Desalting with ion exchange resins and evaporation	Paper chromatography	Schürmann J., Vierteljahrsschrift der Naturforsch. Gesellsch. Zürich 109 409 (1964)

Aminoacids cont.

Compounds	Conc. range	Preconcentration	Method	Authors
16 aminoacids	0.1-17.7 ug/l	Same as Degens (see Appendix I)	Gas chromatography	Peake E., Baker B.L. and Hodgson G.W., Geochim. Cosmochim. Acta 36 867(1972
		Fatty acids		
20 acids	ug/l	Alkaline evaporation of the sample followed by an ether extraction of the acidified residue	Column chromatography and paper chromatography	Mueller H.F., Larson T.E. and Feretti M., Anal.Chem., 32 687(1960)
Formic,	10-24 ug/l	Cation exchange resin for desalting followed by evaporation. The concentrated solution was extracted continuously with diethyl ether	Gas chromatography	Murtaugh J.J. and Bunch R.L., J. Water Poll.Contr. Fed., 37 410(1965)
acetic,	6-72 "			
propionic	0.1-0.8 "			
isobutyric	0.2-1.3 "			
buturic	0.1-0.4 "			
isovaleric	0.1-1.7 "			
valeric	0.1-0.3 "			
caproic	0.3-6.2 "			
	sewage effluents and river water			
13 lower acids	5-590 ug/l	Continuous butanol extraction followed by vacuum evaporation The residue was steam-distillated	Gas chromatography	Lamar W.L. and Goerlitz, U.S., Geol. Survey Prof. Pap., 600-D 24(1968)

Hydrocarbons

Compounds	Conc.range	Preconcentration	Method	Authors
Bitumen-type	0.08-0.1 mg/l	Evaporation	Paper chromatography and fluorometry	Sinelnikov V.E., Kayan. Med. Zh. 3 83(1968)
Alkanes: C_{20}-C_{33}	0.5 ug/l lake waters 1.5 ug/l river waters	Extraction with n-heptane. A few drops of 12 M HCl were added to aid the separation of the layers	Gas chromatography	Peake E. and Hodgson G.W., J. Am. Oil Chem. Soc. 43 215(1966) Peake E., Baker B.L. and Hodgson G.W., Geochim. Cosmochim. Acta 36 867(1972

Humic substances

Humic substances (83% fulvic acids)	3.9 mg/l	Ion exchange with a strongly basic macroporous resin	Elementary analysis	Packham R.F., Proc. Soc. Wat. Treat. Exam. 13 316 (1964)
Yellow organic acids	not given	Ion exchange, freeze concentration, acidification and extraction with butanol	Cryoscopic determination of molecular weight	Shapiro J., J. Am. Water Works Ass. 56 1062(1964)
Humic substances	not given		Sephadex gel filtration	Gjessing E. and Lee G.F., Environ. Sci. Technol. 1 631(1967)
Color producing organic substances	not given	Twenty-fold concentration by vacuum evaporation. Thereafter dialyzing and lyophilizing	Sephadex gel filtration. The fractions were hydrolyzed and investigated on a gas chromatograph	Christman R.F. Symp. Organic Matter in Natural Waters, ed., Hood, D.W., University of Alaska 1970 p. 181

Other organic compounds

Compounds	Conc. range	Preconcentration	Method	Authors
2-methylisoborneol	1.75 ug/l	Ether extraction followed by steam distillation	Gas chromatography-mass spectrometry	Rosen A.A., Mashni C.I. and Safferman R.S. Wat. Treat. Exam. 19 106 (1970)
Geosmin, 2-metylisoborneol	not given	Activated carbon		
Phosphatase amylase saccharase	not given	Direct	Enzymatic and final colorimetric detection	Overbeck J. and Babenzien H.D., Naturvissenschaften 50 571(1963) Reichardt W., Overbeck J. and Steubing L., Nature 216 1345(1967)
About 60 compounds identified up to C_{24}	ng/l level potable and lake waters	Activated carbon filter and circulating systems with purge gas or steam distillation	Gas chromatography mass spectrometry	Grob K. J. Cromatogr. 84 255(1973)

Appendix III

"Dissolved" PCB in different waters

umber of etermin-ations / ype of water	Concentration found ppt	Extraction procedure	Clean up procedure	Confirmation	Authors
2		Reversed liquid-liquid partition. Filter with a nonpolar phase mixture of N-undecane, Carbowax 4000 166 resp. 176 litre pumped through	Fuming sulphuric acid (7% SO_3)	Retention times on different GC columns	Ahling, B. and Jensen, S. Anal. Chem., 42 1483 (1970)
4		Stainless steel screen 16 mesh 30 cm diameter	Silica gel column	Retention times on different GC columns	Duce, R.A. Quinn, J.G., Olney, C.E. Piotrowitz, S.R., Ray, B.J. and Wad T.L. Science 176 161(1972)
rface film d	0.15 and 4.2				
rface sea ter	0.05 and 0.45	Extracted with chloroform			
4					
tuarian water	200-275000	Extraction with petroleum ether	Florisil column	Retension times on different GC columns	Duke, T.W., Lowe, J.J. and Wilson, A.J. Bull. Enviro Contam.Toxicol., 5 171(1971)
4+27		Samples of 19 litre were extracted with 2 l hexane-eter (94:6)	No clean up	Retention times on different GC columns	Harvey, G.R., Ste hauer, W.G. and Teal, J.M. Scienc 180 643(1973)
a water	1-150	Amberlite XAD-2 columns 19-80 litre pumped through			

aknown					
ea water ver water	8-20 2-20	Two litre unfiltered samples were extracted with a mixture of n-hexane and 150 propanol	Silica gel column	Retention times on different GC columns	Holden A.V., Nature 228 1220(1 PCB Conference II Stockholm dec. 14 1972
3					
ver and ke water	3-200	Unknown	Unknown	Unknown	Mayer F.L., Stal ling D.L. and Johnson Y.L. Nature 238 411(19
16					
wage	160-92100	One litre unfiltered samples were extracted twice with 75 ml hexane-15% ether	Florisil column Fuming H_2SO_4-celite saponification KOH	Retention times on different GC columns	Schmidt T.T., Ris brough R.W. and Gress F. Bull. Environ. Contam. Toxicol., 6 235(1971)
14+16	2 max	Activated carbonfilters	Fuming sulphuric acid (10% SO_3)	Retention times on different GC columns	Södergren A. Vatten 2 (1973) Oikos 23 30(1973)
ke water	0.1 max min. not known	Reverse phase filter Some hundred litre were pumped through the filters			
17					
ver water	20-2070	Unfiltered 20 litre samples were extracted twice with 400 ml hexane in 1.6 litre portions	Florisil column	Retention times on different GC columns	Veith G.D. and Lee G.F. Water Res., 5 1107(1972)

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DETERMINATION OF SOLUBLE CARBOHYDRATES IN SEA WATER BY PARTITION CHROMATOGRAPHY AFTER DESALTING BY ION-EXCHANGE MEMBRANE ELECTRODIALYSIS

B. O. JOSEFSSON
Department of Analytical Chemistry, University of Gothenburg, S-402 20 Göteborg 5 (Sweden)
(Received May 25th, 1970)

Reliable methods of characterizing and estimating quantitatively different soluble sugars in sea water do not seem to have been developed, although the presence of dissolved carbohydrates has been shown[1–6]. With a suitable method it would be possible to achieve a better understanding of the microbiological life in the sea and even the effect on the marine environment of human activities.

Since the concentration of soluble carbohydrates in sea water is so low (of the order of μg l^{-1} for each species) it is necessary to isolate them from the sea salts. Isolation techniques have been investigated by JEFFREY AND HOOD[7] and later by BADER *et al.*[8]. They used a ^{14}C-labelled aged algae culture, grown in the laboratory in fertilized sea water to produce dissolved organic compounds. After filtration with Millipore HA (0.45 μm) filters, they tested different methods such as dialysis, electrodialysis with different types of membranes, carbon adsorption, coprecipitation with iron(III) hydroxide, solvent extraction and ion-exchange deionization. They pointed out the difficulties in the quantitative isolation of the organic compounds by these methods. Many compounds, especially carbohydrates, are easily digested by bacterial action. Some of the isolation procedures may cause decomposition and rearrangements of the organic constituents.

DEGENS *et al.*[9] tried a combination of evaporation and extraction with 80% ethanol of the acidified residue followed by ion-exchange desalting to isolate sugars and amino acids from sea water off the coast of California. In this investigation, they found five different monosaccharides, using one-dimensional descending paper chromatography. The quantitative determination was made by visual comparison with synthetic standards, run on the same sheet. The precision in the final stage was about $\pm 15\%$. The isolation procedure however, may cause drastic changes in the organic constituents.

Ion retardation with Retardion 11 A8 was used by SCHAEFER[10] to desalt sea water. This resin is an amphoteric polystyrene, polyacrylate ion-exchanger of so-called "snake cage" type. The ion-exchanger has negative charges ($-COO^-$ from polyacrylic acid), trapped inside a network polymer with charges of the opposite sign ($-R_3N^+$ from Dowex 2-X8). Such a resin swells in water by hydration and absorbs salts much more strongly than non-electrolytes. Thus a column with Retardion 11 A8 will hold back dissolved salts allowing non-electrolytes to emerge from the column first. The separation is especially efficient with rather large molecules of hydrophilic nature such as sugars. With this method it was possible to remove 90%

of the inorganic salts from the "organic fraction" by a one-step procedure. The advantage of this method compared with combined anion and cation ion-exchange is that no regeneration is necessary. It was not possible, however, to remove sulphates completely from the sugars. Our experience in working with low concentrations of glucose in standard sea water is that the desalination is not effective enough, and after evaporation of the eluate it was not possible to retrieve the glucose in the salt residue.

JEFFREY AND HOOD[7] did not report any results from electrodialysis with ion-exchange membranes, but state: "It is believed that under controlled conditions with respect to current, time and membrane arrangement, this technique has unrealized capabilities for the separation of certain organic compounds", *i.e.* from salts.

Demineralisation of sugar solutions was carried out by ANDERSON AND WYLAM[11]. They used electrodialysis with ion-exchange membranes successfully without any loss of sugar and stated that the use of ion-exchange membranes in an electrodialysis cell has definite advantages over other kinds of membranes. Furthermore, they showed that the membranes are impermeable to saccharides and therefore complete recovery of sugars could be obtained from electrodialysed solutions. On the basis of this information a study of desalting by ion-exchange membrane electrodialysis was undertaken in this laboratory; low concentrations of carbohydrates in standard sea water were used, and an apparatus was constructed to fit these conditions. Two methods were used for the quantitative analysis of the sugars in the desalted and evaporated sea water sample, gas–liquid chromatography and liquid–liquid chromatography.

EXPERIMENTAL

Apparatus

The technique of ion-exchange membrane electrodialysis has been described by various workers[12–15].

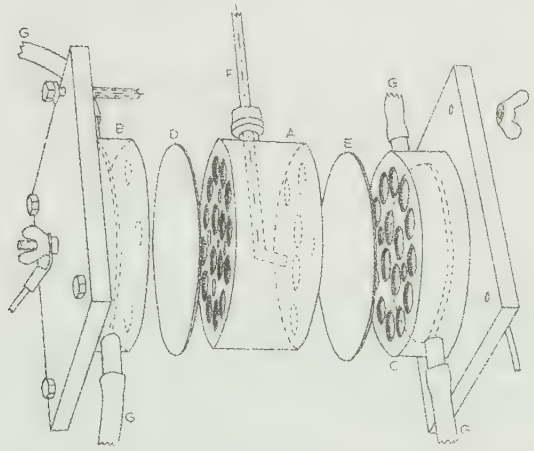

Fig. 1. Exploded view of electrodialysis apparatus. (A) Center compartment for sea water; (B) and (C) anode electrode and cathode electrode compartment; (D) and (E) Permaplex A-20 and Permaplex C-20 ion-exchange membranes; (F) stirrer; (G) flow of tapwater.

The electrodialysis cell used in this work was arranged (see Fig. 1) with three circular compartments. The inner "plexiglas" (Röhm & Haas) walls of the outer compartments and the walls of the inner compartment had matched holes of various sizes (5–20 mm in diameter). The outer walls of the outer compartments consisted of 10 mm thick, 16 × 16 cm square (Plexidur) (Röhm & Haas) plates. The diameter of the compartments was 12 cm and the volume of the inner compartment was 430 cm^3. A second unit has been constructed which holds 2.2 l of sea water. The carbon electrodes were so placed in the outer compartments that they could be cooled by a flow of tap water. The membranes were placed between the compartments, which were pressed together with four screws between the outer "plexidur" plates. Thereby the membranes function as packings, preventing the leakage of both the inner sea-water sample and the outer cooling water.

Membranes

The membranes were Permaplex C-20 and Permaplex A-20 (Permutit Co., Ltd.). These are made of milled synthetic ion-exchange beads with a binder, such as polythene, and extruded in the form of flexible extended sheets. Permaplex C-20 is a cation-selective membrane containing highly ionized sulphonic groups. Permaplex A-20 is an anion-selective membrane containing quaternary ammonium groups. In the fully swollen state they are about 1 mm thick and have very good mechanical strength. The anion-exchange membrane is resistant to attack by chlorine from the anode. The membranes were developed for use in electrolytic cells for removal of ions by electro-osmosis and are therefore permeable to ions of one charge and largely impermeable to ions of opposite charge. They are arranged (see Fig. 1) so that positively and negatively charged ions can pass through the membrane into the cathode and anode compartments respectively.

Power supply

The power supply was a d.c. stabilizer Philips G.M. 455 B which delivers 250 mA at 500 V of direct current. A new power supply which delivers 5 A at 250 V has been constructed.

Electrodialysis cell data

Sea-water sample volume:	430 cm^3
"Effective" membrane surface:	52 cm^2
Current density	5 mA cm^{-2}
Desalting time for 0.5 *M* sodium chloride:	24 h

Sampling of sea water

The sea-water samples were collected from two stations: inside and outside the threshold of the Gullmarfjord on the Swedish west coast. At each station, one sample was taken from the surface layer ($S = 24\,^0/_{00}$) and one from a depth of 35 m ($S = 35\,^0/_{00}$) below the halocline. The water was immediately disinfected with mercury chloride and filtered through a Millipore HA filter with a pore size of 0.45 μm.

Procedure

The sea water was placed in the center compartment of the apparatus. All the

membranes had been soaked and washed before use. A maximum current of 250 mA at a potential of 90 V was applied across the membranes for 24–30 h, depending on the salinity of the sea water (20–35‰). During the electrolysis the potential was increased stepwise to 500 V to maintain a current of 250 mA. At the end of the desalting procedure the current dropped to zero. After desalting, the water was evaporated nearly to dryness at 15 mm Hg and 40°. The residue was then dissolved in 2 ml of 85% ethanol for subsequent analysis by partition chromatography (see below).

Control of salt content

The salt content in the desalted sample was checked in three ways: (*a*) sodium content by atomic absorption spectroscopy, (*b*) conductivity measurements (can also be seen from the current meter on the power supply), (*c*) silver nitrate for the chloride content.

Control of sugar loss

The loss of sugar was studied by a radiochemical technique with D-glucose uniformly labelled with carbon-14 (Radiochemical Centre, Amersham, England). The specific activity was 3.1 mCi $mmol^{-1}$ (17.0 μCi mg^{-1}) of glucose. The β-radioactivity was measured with a windowless, gas flow proportional counter (Nuclear Chicago).

A stock solution was prepared by dissolving 29 mg of D-glucose-^{14}C in 1 l of redistilled water; 10 ml of this solution was diluted with 400 ml of redistilled water. This solution was placed in the middle compartment of the ion-exchange membrane electrodialyzer in the same way as the sea-water samples. A voltage of 200 V was applied for 44 h; 1-ml samples were taken periodically and dried on a planchet for β-counting.

Partition chromatography

The desalted and evaporated sample was dissolved in 2 ml of 85% ethanol. The carbohydrates were then separated by partition chromatography on an ion-exchange column by a method devised by SAMUELSON *et al.*[16,17] with 85% ethanol as the eluant. The column separates saccharides efficiently and the sugars in the eluate were automatically determined in a Technicon Auto-Analyzer by means of an orcinol method. Improvements in this method are being made and will be reported separately.

RESULTS AND DISCUSSION

The desalting procedure

The desalting procedure is highly efficient and no detectable amounts of sodium or chloride can be found. Upon evaporation a small residue is obtained, and this residue may contain other dissolved organic matter than sugars.

The results of the radiochemical determination of the loss of D-glucose-^{14}C are presented in Fig. 2. It can be seen that a slight drop of activity occurs at the beginning, but thereafter the activity is constant. The scatter is most likely due to the difficulty in preparing uniform samples. After 44 h, the remainder of the water, 388 ml (410 ml minus eleven 2-ml samples), was evaporated in the same way as the sea-water samples to 10 ml; 1 ml of this residue was measured for β-activity yielding an activity of 133.500 counts per 18 sec. From the calibration curve which showed that no correction

for dead-time was needed, the glucose loss can be calculated as 11% (133.500 out of 158.500 × 410/388) after corrections for decreased volume.

There is a great advantage with this desalting method which depends on the fact that these sugars are neutral and that they are not influenced to any great extent by the electrical field in the solution. There is no danger of hydrolysis or epimerisation

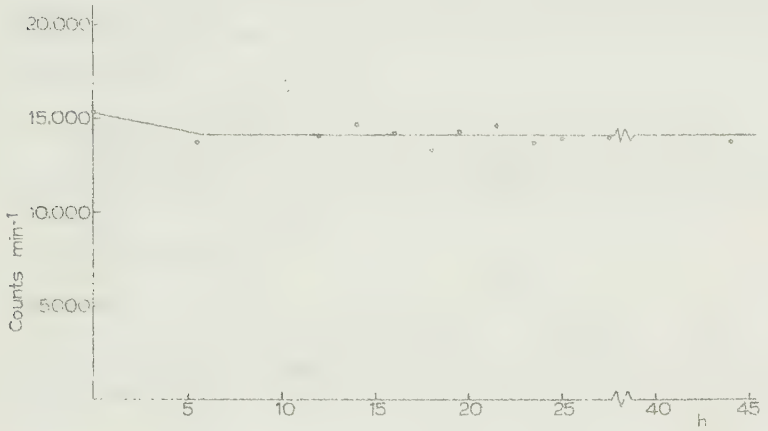

Fig. 2. Activity measurements of ^{14}C-glucose (0.7 μg ml^{-1}) during electrodialysis with a voltage of 200 V applied for 44 h.

of the sugars since the exchange sites of the membranes are not occupied by hydrogen or hydroxyl ions. Unlike the conventional ion-exchangers, the membranes do not require any chemicals for periodic regeneration, because desalting is continuously effected by means of electric power. The time necessary for the desalting operation, in the present case 24–30 h depending on the salinity of the sea water, can easily be reduced with a more powerful rectifier. There is a maximum d.c. voltage or power input for maximum desalting speed depending on the cooling of the electrodes, the gas evolution and the decomposition of the water that can affect the membranes.

The reduction in bacterial count observed in electrodialysis has been reported to be caused by electrophoretic deposition on the surface of the membranes and is favourable for sugar analysis[5]. The sea water is disinfected, of course, but the mercury chloride is removed during the desalting process. The sea-water compartment is closed during the desalting, however, which protects the sugars from bacterial attacks.

Partition chromatography

Partition chromatography on anion-exchange resins in their sulphate form with aqueous ethanol as eluant is a useful tool in the separation of most monosaccharides of biological origin. The results are presented in Fig. 3 and Table I. The percentage error is certainly less than 10% and can probably be lowered to 1%.

Several of the monosaccharides have not previously been found in sea water, but they can now be determined quantitatively. In addition to the monosaccharides presented in Table I, there were indications in some samples of small amounts of fucose and sorbose. The coastal water region in the Gullmarfjord is complicated and

no far-reaching conclusions should be drawn from these few results, which show only the merits of the method.

Chromatographic separation of carbohydrates on cation-exchange resins in the lithium form, confirmed the presence of the reported sugars separated with the anion-exchanger. In addition to the monosaccharides, indications were found that ethylene glycol, glycerol and sucrose were also present. The sample was run in a manner similar to that used with the anion-exchanger partition column. Ethylene glycol and glycerol were determined by acidified periodate oxidation instead of the orcinol method.

Gas–liquid chromatography

Problems arose in the gas chromatographic determination of the sugars from sea water which could not be solved in a satisfactory way. Sugars isolated and dried

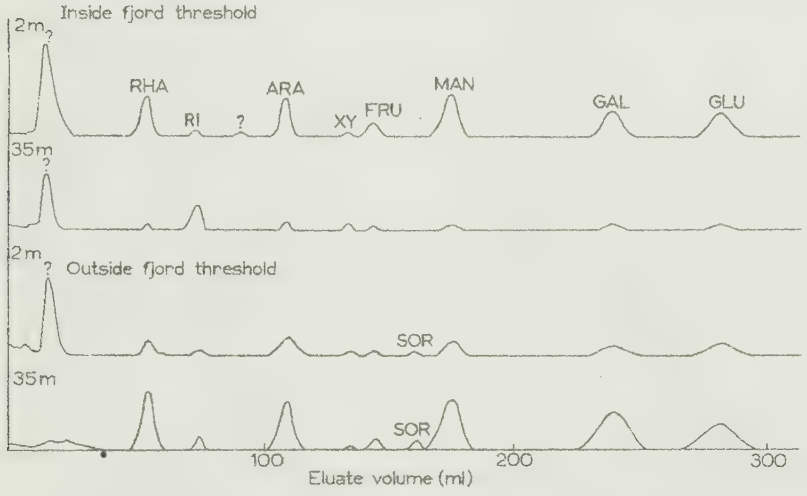

Fig. 3. Liquid–liquid partition chromatogram with anion-exchange column in the sulphate form. Concentrations evaluated from the peak areas are given in Table I.

TABLE I

THE DETERMINATION OF MONOSACCHARIDES IN THE GULLMARFJORD BY LIQUID–LIQUID PARTITION CHROMATOGRAPHY

(All results are given as $\mu g\ l^{-1}$)

	Inside threshold at a depth of		*Outside threshold at a depth of*	
	2 m	*35 m*	*2 m*	*35 m*
Rhamnose	20.5	3.0	5.9	25.2
Ribose	1.0	8.1	0.6	2.8
Arabinose	11.5	3.8	3.9	14.7
Xylose	0.5	2.7	0.3	<0.15
Fructose	14.3	1.2	2.0	2.9
Mannose	29.8	2.7	9.9	32.6
Galactose	32.5	2.7	8.7	37.8
Glucose	46.5	9.3	13.3	32.5

from 2 l of sea water were silylated to trimethylsilylethers[18–28]. The conversion of the carbohydrates to ethers was made with N-trimethylsilylimidazole in pyridine (1.5 meq ml^{-1}). This reagent is the most rapid and powerful silylating agent reported for hydroxyl groups and the reaction is quantitative[28]. It is said[29] that N-trimethylsilylimidazole causes less anomerization than does hexamethyldisilazane and trimethylchlorosilan in pyridine. As can be seen from the chromatograms in Figs. 4 and 5, a number of anomerization peaks appeared and severe overlapping occurred. It is not possible to separate all the peaks on the same column and therefore two columns with stationary phases of different polarity were used. The non-polar phase was 3% OV-1 on (80–100 mesh) Chromosorb W(HP) (Fig. 4) and the mildly polar phase was

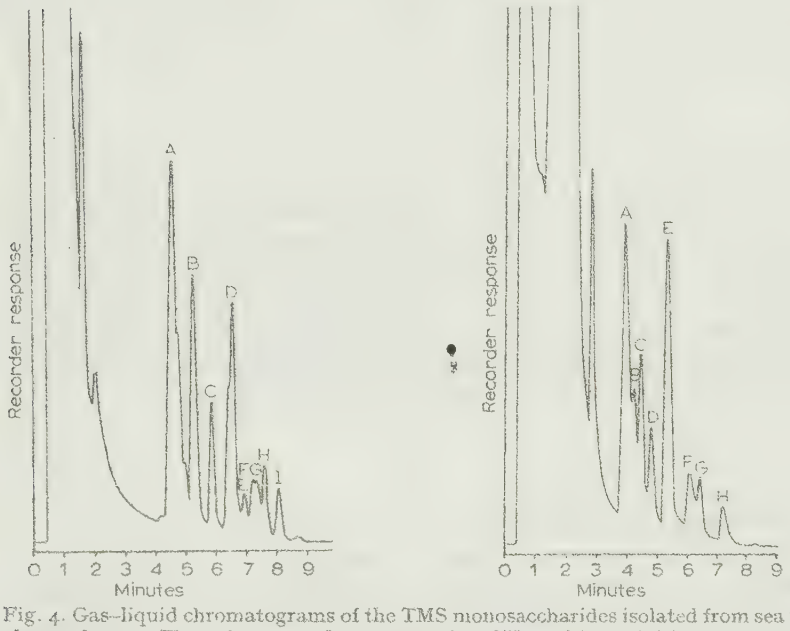

Fig. 4. Gas–liquid chromatograms of the TMS monosaccharides isolated from sea water on 6′ × ¼″ glass columns. The column used was nonpolar OV-1 with an initial temperature of 135° and programmed for a temperature increase of 10° min^{-1}. The carrier gas flow of nitrogen was 20 ml min^{-1}. The components are: (A) rhamnose 1, ribose 1 and ribose 2; (B) rhamnose 2; (C) xylose; (D) fructose, mannose 1 and galactose 1; (E) galactose 2; (F) mannose 2; (G) galactose 3; (H) glucose 1; (I) glucose 2.

Fig. 5. Gas–liquid chromatograms of the TMS monosaccharides from the same sample as in Fig. 4. The conditions were the same but mildly polar columns OV-17 were used. The components are: (A) rhamnose 1, xylose 1; (B) ribose; (C) rhamnose 2; (D) fructose; (E) mannose 1, galactose 1; (F) galactose 2; (G) glucose 1, galactose 3; (H) glucose 2.

3% OV-17 on (80–100 mesh) Chromosorb W(HP)(Fig. 5). The separation was done on a Perkin-Elmer 881 dual-column gas chromatograph fitted with a hydrogen flame ionisation detector. The samples were run with temperature programming, 10° min^{-1}, on coiled glass columns with nitrogen as carrier gas and a flow rate of 20 ml min^{-1}. Because of serious overlapping of the different anomers, it was not possible in all cases to identify the form of mutarotation. Overlapping also prevented the identification of arabinose.

Gas–liquid chromatography methods cannot be used properly as a quantitative procedure for carbohydrates in sea water. There are too many carbohydrates with complex biological origin. Reduction of sugars to sugar alcohols reduces the number of multiple peaks caused by anomerization[30], but the loss of material by reduction is considerable, and some sugars are reduced to the same alcohol (*e.g.* sorbose and glucose both give sorbitol). Furthermore, the separation of hexitols is very poor[18,21].

The author expresses his thanks to Professor DAVID DYRSSEN for valuable discussions, to Professor OLOF SAMUELSON, Department of Engineering Chemistry, Chalmers Institute of Technology, for the use of the liquid–liquid partition chromatographic apparatus, to Dr. OLLE RAMNÄS for modifying this apparatus for the samples prepared from sea water, and to Professor JAN RYDBERG for the use of radiochemical apparatus. This work was supported by the Swedish Natural Research Council.

SUMMARY

A method for the determination of different soluble sugars in sea water is discussed. An apparatus for desalting the sea water by electrodialysis with ion-exchange membranes is described. The desalted samples are evaporated *in vacuo* and investigated by liquid–liquid and gas–liquid chromatographic methods. These methods are compared and the former is shown to be superior for the determination of different sugars. With the liquid–liquid partition chromatographic method, eight monosaccharides were determined in the range of 0.15–46.5 μg l^{-1}; small amounts of sorbose, fucose, sucrose, ethylene glycol and glycerol were also detected.

RÉSUMÉ

Une étude est effectuée sur le dosage de différents sucres solubles dans l'eau de mer. On décrit un appareil pour désaler l'eau de mer par électrodyalise avec des membranes échangeuses d'ions. Les échantillons désalés sont évaporés au vide et examinés par dés méthodes chromatographiques liquide–liquide et gaz–liquide. Par la méthode chromatographique de partage liquide–liquide, on a pu doser huit monosaccharides à des concentrations de 0.15 à 46.5 μg l^{-1}; de failles teneurs de sorbose, fucose, sucrose, éthylèneglycol et glycérol peuvent être également détectées.

ZUSAMMENFASSUNG

Es wird ein Verfahren zur Bestimmung verschiedener löslicher Zucker in Meerwasser erörtert. Eine Apparatur für die Entsalzung von Meerwasser durch Elektrodialyse mit Ionenaustauschmembranen wird beschrieben. Die entsalzten Proben werden im Vakuum eingedampft und mit Methoden der Flüssig–Flüssig- und Gas–Flüssig-Chromatographie untersucht. Diese Verfahren werden miteinander verglichen; das erstere ist für die Bestimmung verschiedener Zucker besser. Mit der Flüssig–Flüssig-Verteilungs-chromatographie wurden acht Monosaccharide im Bereich 0.15–46.5 μg l^{-1} bestimmt; kleine Mengen von Sorbose, Fucose, Sucrose, Äthylenglykol und Glycerin wurden ebenfalls nachgewiesen.

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INSTRUMENTS AND METHODS

Automatic spectrophotometric procedures for the determination of the total amount of dissolved carbohydrates in sea water

BJÖRN JOSEFSSON,* LEIF UPPSTRÖM* and GÖRAN ÖSTLING*

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Abstract—Five different reagents for carbohydrates, anthrone, phenol, orcinol, N-ethylcarbazole and L-tryptophan have been tested for their possible use in automatic procedures for the determination of the total amount of dissolved carbohydrates in sea water utilizing the Autolab equipment. In the investigated range, 0–4 mg carbohydrate per litre, all the reagents except phenol could be used for analysis with the standard outfit. Due to the fact that the various sugars tested give the least spread spectra and calibration curves with L-tryptophan and that no salinity dependence was found, the use of that reagent is recommended.

FOR INVESTIGATION of the natural content of organic substances in the sea or studies of sea water polluted by dumped organic compounds a screening process undertaken with only short time delay is desirable. Examination over large areas and different depths are necessary to give the seasonal variation background. In a review dealing with the composition of dissolved organic compounds in sea water WAGNER (1969) concluded that procedures have yet to be developed that are sufficiently simple, reliable, fast and accurate enough to permit widespread, systematic surveying of the various organic components. The need for automatic systems in such work is obvious. The advantage of carrying out the analyses on board ship whereby preservation and storage of samples can be avoided is evident.

One main aspect of studying the organic constituents of natural waters is the possibility of increasing our knowledge of the ecology of living organisms. In such studies the estimation of the dissolved carbohydrates is of great importance. Work has previously been performed to measure the total amount of dissolved carbohydrates given in glucose units, or any other arbitrarily chosen scale, to relate the different samples. The dissolved carbohydrates are thereby simply defined as those carbohydrates able to pass through a Millipore® HA (0·45 μm) filter and characterized by reaction with reagents like anthrone or N-ethylcarbazole. It is also understood that certain substances which are not generally classified as carbohydrates are included due to their positive test reactions.

A few authors report successful isolation and specific determination of particular sugars in sea water. DEGENS, REUTER and SHAW (1964) found glucose, mannose and galactose in sea waters off the Californian coast. VACCARO, HICKS, JANNASCH and CAREY (1968) estimated the glucose content in various Atlantic sea waters utilizing a specific enzymatic method developed by HICKS and CAREY (1968). Recently JOSEFSSON (1970) was able to determine eight monosaccharides quantitatively as well as to identify some other organic components by an analytical procedure, where the isolation was accomplished through desalting of the water by electrodialysis with ion exchange membranes, followed by evaporation *in vacuo* and liquid–liquid chromatography. The time required for such an analysis, mainly depending on the desalination and evaporation processes, makes it unsuitable for 'on board' determinations. If a detailed establishment of the various components is required, samples must still be preserved and stored for later examination.

*Department of Analytical Chemistry, University of Gothenburg, Fack, S-402 20 Göteborg 5, Sweden.

PREVIOUS WORK ON REAGENTS INVESTIGATED

In this work, three well-known analytical reagents for carbohydrates, anthrone, N-ethylcarbazole and phenol as well as two other reagents for sugars, orcinol and L-tryptophan were investigated, for application to automatic procedures for the determination of the total amount of dissolved carbohydrates in sea water.

The use of anthrone goes back to a work by DREYWOOD (1946) and many methods are based on this reagent. The use of anthrone for sea water analyses was studied by ANTIA and LEE (1963). A modification of the anthrone method developed by STRICKLAND and PARSONS (1965) was employed by BIGGS and WETZEL (1968) in an investigation of the vertical distribution of particulate carbohydrate in Chesapeake Bay to study the effectiveness of the halocline as a barrier to sinking organic material. They did not make a separate determination of the dissolved carbohydrate which might have given further interesting information. The anthrone method has also been used by KAWAHARA, MAITA and ISHII (1967) for determination of dissolved carbohydrates in the waters of Southern Hokkaido, and by SUGAWARA and KAMATA (1965) in a study of the vertical distribution of dissolved carbohydrates in Lake Kizaki-ko, Japan. N-ethylcarbazole was first used as a reagent for carbohydrates in sea water by ERDMAN and LITTLE (1950). The method was further studied and improved by ZEIN-ELDIN and MAY (1958).

In a paper by HANDA (1966) the applicability of the phenol sulphuric acid method to sea water analysis is discussed. The work is based on the observation by DUBOIS, GILLES, HAMILTON, REBERS and SMITH (1956) that quantitative micro-determination of monosaccharides, oligosaccharides and polysaccharides can be performed by the colour obtained when phenol is used as a chromophore in strong sulphuric acid solution. Handa compared the phenol method with the N-ethylcarbazole and anthrone methods and found it free from interferences when hydrazine sulphate or stannous chloride was added to the sulphuric acid. The investigation is, however, not performed in the concentration range usually expected for the total amount of dissolved carbohydrate in sea water.

Orcinol and L-tryptophan have to our knowledge not so far been applied to direct sea water analysis. Orcinol has, however, been used in an automated procedure by LARSSON and SAMUELSON (1967), for the determination of monosaccharides in biochemical work. They also used it for analysis of the carbohydrate composition in rayon pulp, alkali cellulose and rayon (1968). In a method for the determination of plasma polysaccharides BADIN, JACKSON and SCHUBERT (1953) used L-tryptophan in combination with a solution of boric acid in sulphuric acid. According to these authors the colour density produced by different sugars are nearly the same which would make the L-tryptophan reagent ideal for the estimation of the total amount of dissolved carbohydrates in sea water.

AUTOMATIC SYSTEM

The equipment used for the automatic procedures in this work is the so called 'Autolab', previously described by HULTHE, UPPSTRÖM and ÖSTLING (1970) for the determination of boron in sea water. Tubes of glass or polythene containing the filtered sea water samples are placed in holders linked together forming an outer chain. A pipetting device automatically transfers a preset sample volume into glass tubes in an inner processing chain. The sets of tube holders can be connected to form a chain of any length, and thus function as a time regulator. The chain passes the modules chosen for the analyses. Values of absorbance are printed on paper tape in an arbitrarily chosen scale unit, in this paper denoted A_{arb}, which, if necessary, can be transformed to common units. For technical reasons the background absorbance is not eliminated in the calibration curves obtained. The background value, which varies somewhat, was not determined for all series and has thus been left out in the calibration curves. The methods mentioned above are the basis for the following procedures to be described though they are modified depending on the practical limitations of the equipment, e.g. time and volume restrictions.

EXPERIMENTAL

Apparatus

An Autolab equipment (AB. Lars Ljungberg & Co., Stockholm, Sweden) with the following modules was used: 1 sampleboard, 1 sample transfer unit, with a specially made teflon pipette, 1 time delay board, 3 dispenser units, 1 thermostatted oil bath, 1 interference filter photometer with a standard 1 cm cell and a band-width of 12 nm. As heating medium silicon oil is to be preferred. In the N-ethylcarbazole method, where air oxidation of the samples must be avoided, two mechanical stirrers were replaced by nitrogen gas bubbling jets. Necessary filtrations were made by a Millipore® equipment with filters HA 0·45 μm. A Beckman DB spectrophotometer was used for the scanned absorption spectra.

Reagents

Anthrone reagent. Dissolve 1 g of anthrone (for chromatography, Merck AG, Darmstadt) in 1 litre of sulphuric acid, s.g. 1·81.

N-ethylcarbazole reagent. Dissolve 2 g of N-ethylcarbazole, (Th. Schuchardt, München) three times recrystallized in warm water–ethanol solution, in 1 litre of sulphuric acid, s.g. 1·81.

Phenol reagent. Dissolve 50 g of phenol in redestilled water.

Hydrazine sulphate solution. Dissolve 5 g of hydrazine sulphate in 1 litre of sulphuric acid, s.g. 1·81.

Orcinol reagent. Dissolve 5 g of orcinol (cryst., Merck) in 1 litre of sulphuric acid, s.g. 1·81.

L-*tryptophan reagent.* Dissolve 25 g of boric acid and 5 g of L-tryptophan (CHR, puriss., Fluka AG, Switzerland) in 1 litre of sulphuric acid, s.g. 1·81.

If not otherwise stated the reagents used in this work are of analytical grade from Merck AG, Darmstadt. All reagents must be freshly prepared.

Composition of samples

The samples investigated were made by addition of different sugars to Copenhagen standard sea water or in the case of salinity dependence synthetic sea water according to LYMAN and FLEMING (1940). After filtration the sugar was added and all samples were sterilized by the addition of mercuric chloride and stored in a refrigerator.

General procedure

Calibration curves for different sugars in the concentration range of 0·5–4·0 mg/l. were obtained for each of the tested reagents. Each point is the mean value of five replicate sample results. The reaction time in the oil bath was 17 minutes at a bath temperature of 100 ± 2° except for the N-ethylcarbazole method where 13 minutes at 75 ± 2° was found to be more suitable, and the phenol method where a reaction time of 16 minutes at room temperature is sufficient. Detailed reaction schemes are sketched in Fig. 1. The response time for these methods is less than 35 minutes. The salinity dependence has been investigated in the concentration range of 5–35 ‰. In Autolab procedures the transfer of the samples from the tubes in the input chain to the reaction tubes is usually made by rinsing the sampling pipette with the first reagent solution, thus assuring that the sample volume is constant. As the first reagents in these methods contain concentrated sulphuric acid, which already in the pipette vigorously reacts with the aqueous solution, a new technique must be introduced. Rinsing with water would give an inconvenient dilution, but the use of an inert hydrophobic organic phase of less density than the sample permits elution with only a small volume. In this work n-heptane (Mallinckrodt, U.S.A.) was used.

The coefficient of variation was determined for the sugars yielding the strongest absorbance with the different reagents, except phenol, and can be found in Table 1.

Table 1. *Standard deviation values obtained for sugar determination with the Autolab equipment.*

Reagent	*Sugar tested*	*Concentration* (mg/l.)	*Number of samples*	*Coefficient of variation* (%)
Orcinol	Arabinose	2	64	2·6
	Glucose	2	24	6·0
Anthrone	Fructose	2	35	7·6
	Glucose	2	35	4·0
L-Tryptophan	Fructose	2	20	3·3
	Glucose	2	20	1·3
	Arabinose	2	20	2·4
N-Ethylcarbazole	Fructose	2	24	2·9
	Glucose	2	25	5·3
	Arabinose	2	25	3·3

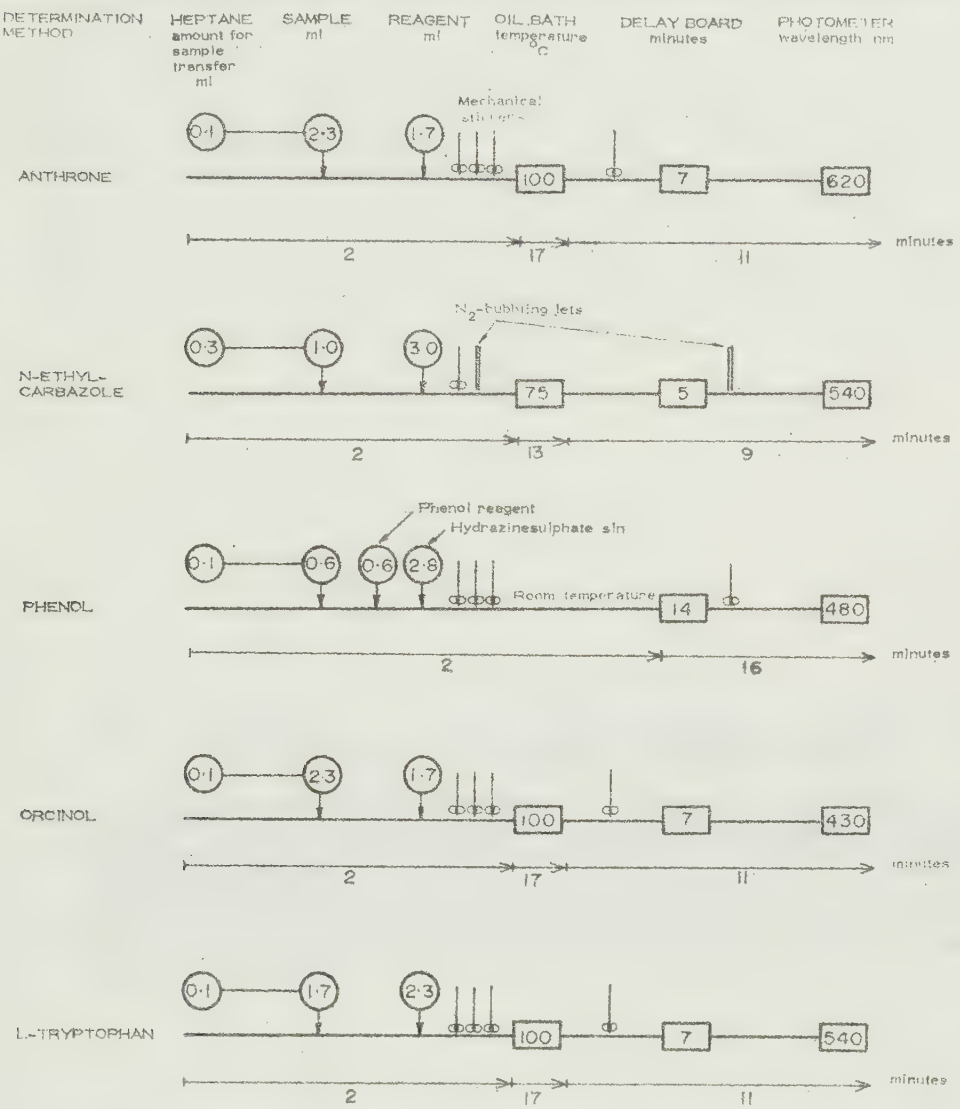


Fig. 1. Working schemes for the different methods applied to the Autolab equipment.

RESULTS

The results for the different reagents and types of carbohydrates are presented in two ways: absorption spectra (Figs. 3, 5, 7 and 10) scanned with a Beckman DB spectrophotometer and calibration curves (Figs. 2, 4, 8 and 9) measured with the Autolab photometer at selected wave-lengths. The band widths of the two photometers are 2 and 12 nm, respectively. The results for each reagent are treated separately.

The anthrone method

1·7 ml of the anthrone reagent was added to 2·3 ml of the sample and the solution then mixed by mechanical stirring. The further treatment is described in Fig. 1. Measurements were performed at 620 nm. Figure 2 shows the calibration curves obtained. The absorbance curves in Fig. 3 show the relative absorbance for the same weight amounts of the different sugars. Thus, for example, it can be

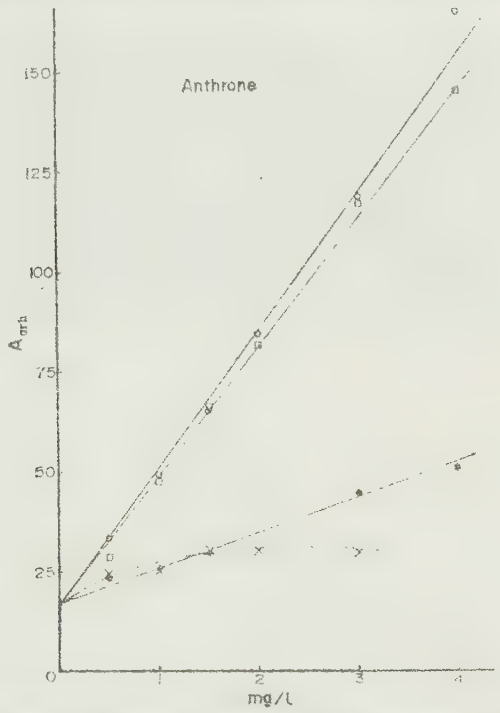


Fig. 2. Calibration curves for fructose (○), glucose (□), rhamnose (•) and arabinose (x) with the anthrone reagent. Absorbance (A_{arb}) in an arbitrarily chosen scale.

seen that galactose would have given a calibration curve between glucose and rhamnose but near to the latter. The anthrone reaction as described here gives mainly the sum of glucose, fructose and smaller contributions from other carbohydrates like galactose and rhamnose. Interferences from other organic compounds e.g. methionine and tryptophan as well as inorganic species such as nitrates may be important and are discussed in the work by ANTIA and LEE (1963). We found that for a salinity variation between 5–35 ‰ the calibration curves were not affected. This confirms the results by ANTIA and LEE (1963). The salinity dependence they found in the concentration range of 0–5 ‰ must be considered if low salinity waters are to be analysed.

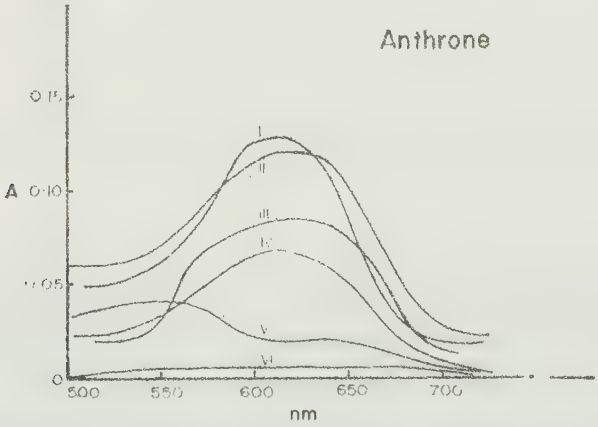


Fig. 3.. Absorbance spectra for the reaction products of carbohydrates with anthrone. Sugar concentrations: 8 mg/l. I glucose, II fructose, III galactose, IV mannose, V rhamnose and VI arabinose.

The N-ethylcarbazole method

A sample of 1·0 ml was taken with the aid of 0·3 ml of heptane, and 3·0 ml of the reagent was added and after stirring nitrogen was bubbled through the solution at two different stages (see Fig. 1). The reaction solutions must be protected from direct light to guarantee good results according to ZEIN-ELDIN and MAY (1958). The bath temperature should not exceed 75° for the reaction time chosen to prevent sudden colour changes which appear in more heated solutions. Thus the reaction is very much depending on at least four parameters: dissolved oxygen content, light, temperature, and reaction time. The difficulty in controlling these parameters makes the method somewhat unreliable, especially in its manual version. Much of these disturbances has been discussed in the paper by ZEIN-ELDIN and MAY (1958) who also showed that the sulphuric acid must be pre-tested. The calibration curves at 540 nm (Fig. 4) and spectra (Fig. 5) show that the absorbance contributions from the various sugars are rather similar and values of total dissolved carbohydrate in e.g. glucose units give a rather good mean value of the total carbohydrate content, provided that the concentration ratios of the different

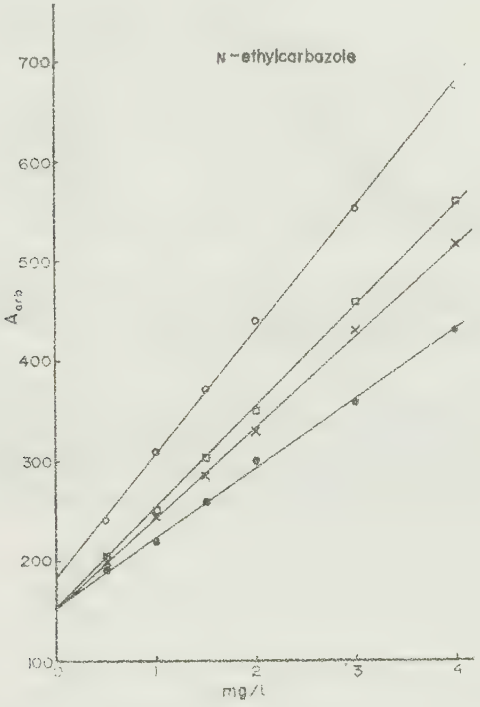


Fig. 4. Calibration curves for fructose (○), glucose (□), rhamnos (•) and arabinose (x) with the N-ethylcarbazole reagent. Absorbance (A_{arb}) in an arbitrarily chosen scale.

sugars present are those reported by JOSEFSSON (1970). The differences between the absorption curves are smaller in our procedure than those found by HANDA (1966). This is probably due to the different temperatures used and in our opinion the lower temperature gives less spread in the calibration curves (Fig. 4). The salinity dependence was tested for three sugars: fructose, glucose and arabinose and can be seen in Fig. 6. It was found that the absorption increases with decreasing salinity in the range of 5–35 ‰. This shows that the calibration solutions must have the same salinity as the sample if correct values should be obtained or else a salinity correction factor must be introduced. Contributions from other organic species than carbohydrates have not been furnished by the authors mentioned above. MCLAUGHLIN, ZAHL, NOWAK, MARCHISOTTO and PRAGER (1960), however, reported interfering non-carbohydrate materials.

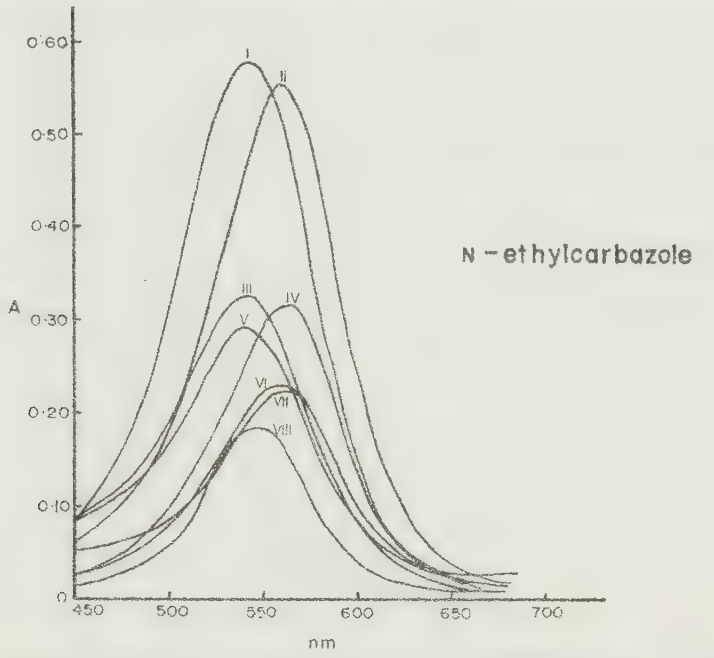


Fig. 5. Absorbance spectra for the reaction products of carbohydrates with N-ethylcarbazole. Sugar concentration: 8 mg/l. I xylose, II fructose, III ribose, IV glucose, V arabinose, VI galactose, VII mannose, VIII rhamnose.

The phenol method

To a sample volume of 0·6 ml was first added 0·6 ml of the phenol solution and then 2·8 ml of the hydrazine sulphate solution and the mixture was well stirred. After 16 minutes at room temperature the absorbance at 480 nm was measured. For the concentration range used by HANDA (1966) excellent results were obtained. However, in the range of interest, that is 0–4 mg carbohydrate/l., the method was found to be too insensitive, with a high standard deviation. If longer cells could be used with

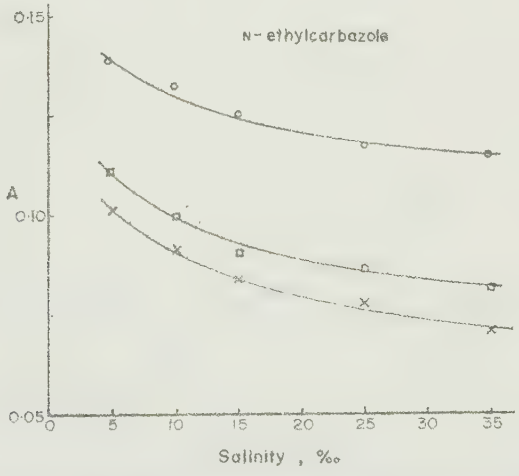


Fig. 6. Salinity dependence of the reaction between N-ethylcarbazole and carbohydrates. The solutions were made of synthetic sea waters and the sugar concentration was 2 mg/l. Fructose (○), glucose (□) and arabinose (x) were tested.

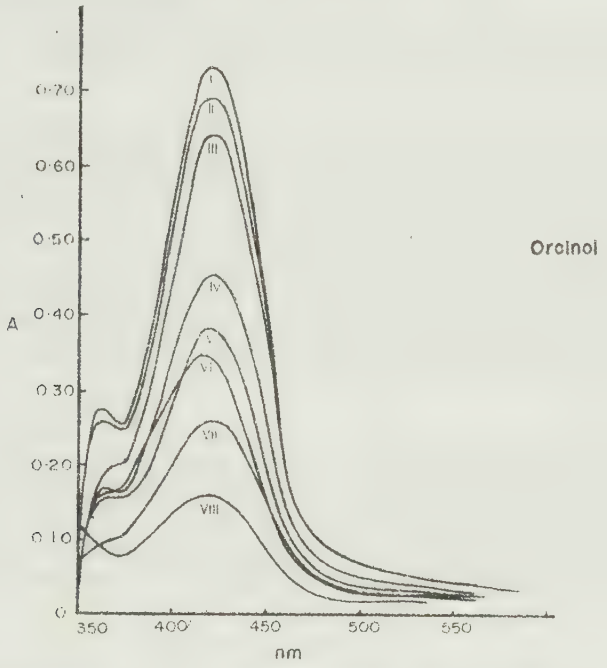


Fig. 7. Absorbance spectra for the reaction products between orcinol and carbohydrates. I ribose, II xylose, III arabinose, IV mannose, V galactose, VI rhamnose, VII glucose, VIII fructose. Sugar concentration 4 mg/l.

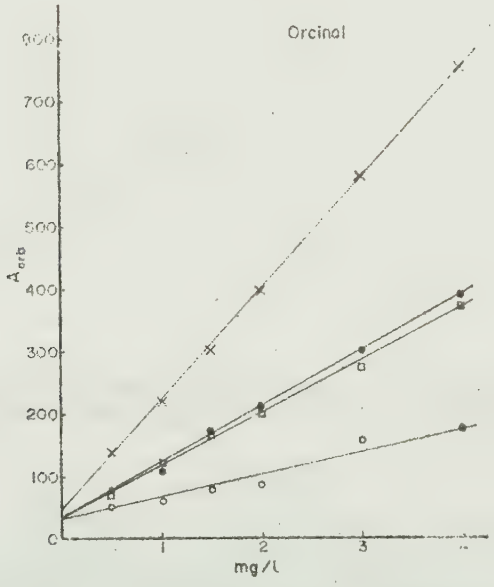
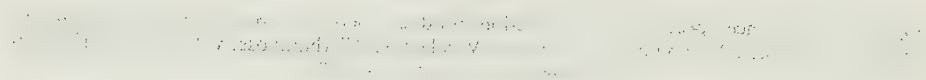


Fig. 8. Calibration curves for arabinose (x), rhamnose (•), glucose (□) and fructose (○) with the orcinol reagent.



e.g. 5 cm path length this difficulty could be overcome and direct measurements applied. The equipment used could in our opinion easily be reconstructed to fulfil this requirement. Some other results (e.g. salinity independence) found by HANDA (1966) were confirmed and need not be repeated here.

The orcinol method

To a sample volume of 2·3 ml was added 1·7 ml of the orcinol reagent and the reaction solution was three times stirred. The temperature in the oil bath was held at 100° and the absorbance measurements performed at 430 nm. As can be seen from the spectra in Fig. 7 and the calibration curves in Fig. 8, the resulting absorbance differs widely between the sugars analysed. Orcinol is most sensitive to the pentoses ribose, xylose and arabinose and small amounts (0·1 mg/l.) are easily detected. Glucose and rhamnose show considerably lower absorbance values, and fructose gives a very weak colour reaction. Thus the scatter of the resulting absorbances give difficulties in estimating a good mean value of the total amount of dissolved carbohydrates. The reagent is practically independent of the salinity in the region 5–35 ‰. For the analysis of pure arabinose solutions the reagent is excellent and for concentrations around 2 mg/l. a coefficient of variation of 1·3 % was obtained. Orcinol is used for the qualitative and quantitative determination of sugars in the work by JOSEFSSON (1970).

The L-tryptophan method

To a volume of 1·7 ml sample solution was added 2·3 ml of the tryptophan reagent. The reaction temperature was kept at 100°. Measurements were made at 540 nm and the resulting calibration curves can be seen in Fig. 9. All the curves show nearly the same slope within the concentrations range investigated and a sensitivity high enough to allow a satisfactory analysis. Figure 10 shows that the absorbance curves for the different sugars are more collected than for the other reagents mentioned in this paper. The coefficient of variation was found to be below 3·3 % for the tested solutions (see Table 1). No salt dependence could be detected in the range 5–35 ‰ salinity.

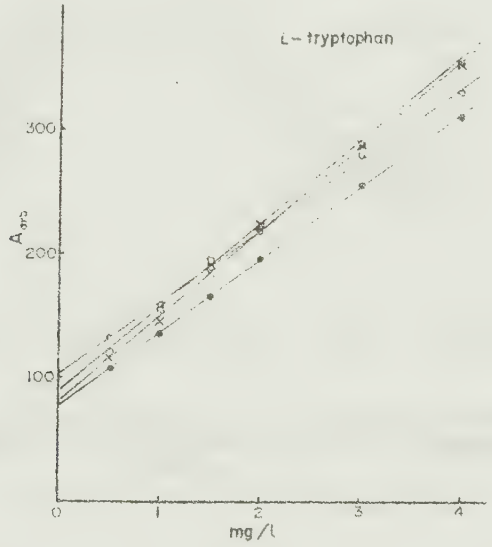


Fig. 9. Calibration curves for glucose (□), arabinose (x), fructose (○) and rhamnose (•) with the L-tryptophan reagent. The curves are almost parallel and adjacent.

DISCUSSION

All the reagents investigated except phenol can be used for direct determinations of the total content of dissolved carbohydrates in small amounts in sea water with the Autolab equipment. For very low sugar concentrations (in the range of 0·05–0·1 ppm) longer cuvettes (e.g. 5 cm) should be applied. The mixing processes are better reproduced in automatic procedures and are especially advantageous for the anthrone and N-ethylcarbazole reagents, for which the manual methods include an awkward temperature control as well.

The necessary breakdown of the carbohydrates in the methods described is performed with strong sulphuric acid. The acid causes dehydration of monosaccharides in a variety of ways depending upon the reaction conditions and the nature of the carbohydrate. Pentoses give mostly furfural and hexoses give generally hydroxymethyl furfural. However, many other degradation and polymerisation products are produced by the acid treatment of sugars. Degradation products such as γ-pyrone and benzene derivatives have been detected. This explains partly the complexity of the colour reactions.

A weakness in most carbohydrate determination methods is that the measurements are relative, that is, the values are estimated in relation to one sugar of general occurrence e.g. glucose or arabinose. The results depend on the reagent used. This makes the various values obtained by different workers difficult to compare. Orcinol, for instance, is very sensitive to pentoses but moderate to ketohexoses and aldohexoses and consequently a low value compared to an arabinose standard could have a relatively high value in comparison to glucose. The fact that the different sugars tested give rather similar absorbance curves with the reagent L-tryptophan is of great advantage as any common sugar

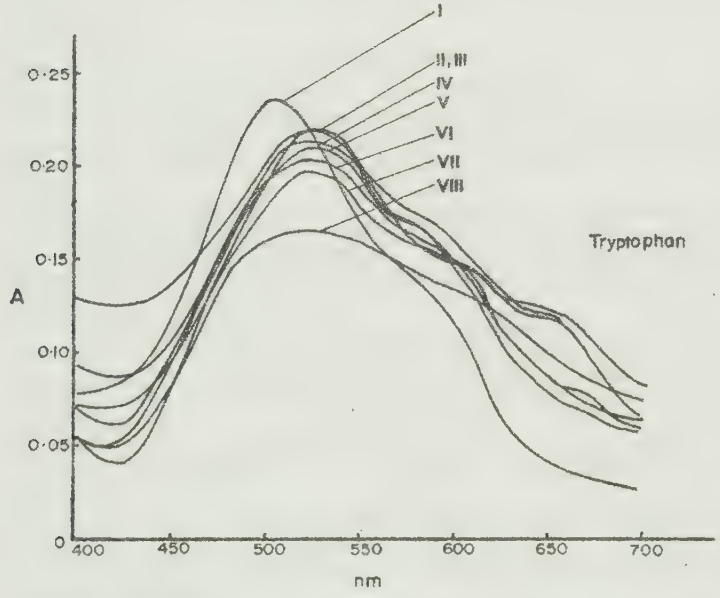


Fig. 10. Absorbance spectra for the reaction products of L-tryptophan and different carbohydrates. I rhamnose, II xylose, III glucose, IV arabinose, V galactose, VI mannose, VII ribose, VIII fructose.

may be used for the calibration curve and the resulting values are still comparable. This means that there is no important obliquity in the absorbance values depending on the various types of carbohydrates present in the samples. As there is no salt dependence and the analytical technique is comparatively simple, the L-tryptophan method is recommended, although we are aware that the reagent must be further tested for the establishment of possible interferences.

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A fluorimetric determination of lignin sulfonates from natural waters in the presence of humic substances

TOM ALMGREN and BJÖRN JOSEFSSON,
University of Gothenburg, Department of Analytical Chemistry, Göteborg

KEYWORDS: Lignosulfonates, Humic materials, Spectrochemical analysis, Chemical analysis, Water pollution.

SUMMARY: Different methods for the determination of lignin sulfonates in the presence of humic substances are discussed. It is shown that the UV absorbance of humic substances in general will conceal the absorbance of lignin sulfonates. Using water from the river Nissan it is, however, shown that the introduction of lignin sulfonates from a paper and pulp mill may be measured spectrofluorimetrically. The sensitivity of 0,2 ppm lignin sulfonates in the presence of naturally occurring humic substances can probably be increased to 20 ppb. The principles for a simple field fluorimeter are outlined.

The suggested method is based on measurements of the fluorescence spectra of two different lignin sulfonates, Björkman lignin, and humic substances in the river Nissan and isolated from the Göta river. There are definite differences in peak wave-length and emission intensity per weight per volume. The fluorescence of waters from the Byfjord and the Hanö bay are very similar and resemble mainly the spectra of humic substances.

ADDRESS OF THE AUTHORS: Fack, 402 20 Göteborg 5, Sweden

Organic compounds are introduced into the sea from many and varied sources. The spent sulfite liquors from pulp mills discharged in different waters have been of major interest. These pollutants have a high oxygen demand and may thereby cause damage to the fish resources amongst other deleterious effects. Thus, analytical methods for the detection and the quantitative determination of lignin sulfonates in both sea water and fresh water are of considerable importance.

Different methods are used to determine organic pollutants. The most common methods seem to be different oxygen demand determinations as biological oxygen demand (BOD) and chemical oxygen demand (COD). These methods, however, are not specific for lignin sulfonates, but reveal sources of pollution and are well employed since many decades. Other methods used for the determination of lignin sulfonates are based on UV spectrophotometry. Hedlund and Wilson (1) found that lignin sulfonates could be determined in fresh waters by determining the difference in UV absorbance at 265 and 280 nm. They had to make a correction for natural fresh waters containing humic substances. This method is suitable for high amounts of spent sulfite liquors. However, the sensitivity is poor (abot 1 mg/l) and the method can not be used for sea water. Lindberg (2) employed a similar method, but the solution was first measured at 250 nm in neutral solution and thereafter at 280 nm in alkaline solution (pH 12). The humic substances are said to cause no disturbances. However, it is not possible to make sea water alkaline without precipitation of magnesium hydroxide. For brackish water Koroleff (3) has proposed to complex magnesium and calcium with citrate in order to reach as high pH as possible without precipitation. For sea water this complexing was not sufficient to avoid partial precipitation. The use of the methods mentioned above are hampered by the presence of other products such as sewage, other industrial waste and dissolved natural compounds.

A more specific method for quantitative determination of lignin sulfonates and lignin in sea water were proposed by Pearl and Benson (4, 5). Polyphenolic substances react with nitrite in acidic solution to form nitroso derivatives, which in alkaline solution, tautomerize to more highly colored quinone oxime compounds. In addition to lignin sulfonates other phenols such as kraft lignins, tannins and certain inorganic substances may also react with nitrite to give colored substances according to Mohler and Jacob (6). Hydrogen sulfide, iodide and iron (II) salts may be oxidized and give brown vapors of nitric oxide. The reactions of amines and nitrogen containing substances with nitrous acid have been studied quite extensively and give diazotised colored products. Because of all these interferences with non-lignin sulfonates the results are often reported as "apparent spent sulfite liquor". Even though the Pearl-Benson method is simple and has a rather good sensitivity, the detection limit being about 0.3 mg/l, it is not specific enough. Felicetta and McCarty (7), suggested a more specific, but rather time-consuming, method to determine the lignin sulfonates in natural waters. The method was based on the cleavage reaction of lignin sulfonates with alkali yielding vanillin. The vanillin was extracted and determined by aid of paper or gas chromatography. Even this method has been criticized as being not unique for lignin sulfonates because naturally occurring wood extractives give vanillin upon alkaline cleavage.

Fluorimetric detection of lignin sulfonates in natural waters were investigated by Christman and Minear (8). They studied fundamental parameters such as optimum excitation and emission frequencies and characteristics for different molecular-weight fractions of lignin sulfonate waste. Environmental factors such as pH, temperature and salinity on these parameters were also investigated. They found a better sensitivity than with the Pearl-Benson procedure and linear relationships in the concentration ranges 1—5, 0.5—5 and 0.2—1.5 ppm PB (Pearl Benson Index, 10 percent total solids) for different excitation frequencies. The method eliminated time-consuming calculations of the final concentration. Fluorimetric studies of dissolved organic matter in sea water have been made by Lorenzen (9) and by Traganza (10). Lorenzen used an instrument for continuous detection by pumping techniques. Baumgartner et al. (11) proposed a fluorescence method for direct tracing of kraft mill effluent from an ocean outfall instead of using rhodamine dye. Wilson (12) determined the fluorescence spectra for fresh water dilutions of spent kraft liquor. He was able to show differences in fluorescence spectra for liquors from Hemlock batches and Douglas fir batches.

Differences in fluorescence spectra related to changes in cooking processes showed that an absolute determination, uniformly applicable to all these effluens, was difficult and had to be studied more exhaustively. Humic acids and other interfering polyphenolic substances do not seem to have been studied fluorimetrically before. As humic substances are very common in Scandinavian waters we wished to investigate if it was possible to use

direct fluorimetry for routine determination of lignin sulfonates in different natural waters.

Experimental

Apparatus

For the most precise and exhaustive flourescent analyses, instruments employing two monochromators are preferred. The Aminco-Bowman Spectrofluorimeter, a single beam instrument, was used in this work. It has two Czerny-Turner grating monochromators with a wavelength drive mechanism on the input energy and a reorder on the output fluorescent energy. Thus, fluorescent patterns can be scanned rapidly over wide ranges of excitation energies and optimum conditions can easily be obtained. The excitation energy peaks were localized by manual scanning. An air-cooled xenon-mercury high pressure lamp was used giving intense continuous spectra from 200 to 750 nm. No excitation correction accessory was provided. Solution samples were measured in a 1 cm quartz cell. Absorbtion spectra were determined on Beckman DK2A spectrophotometer.

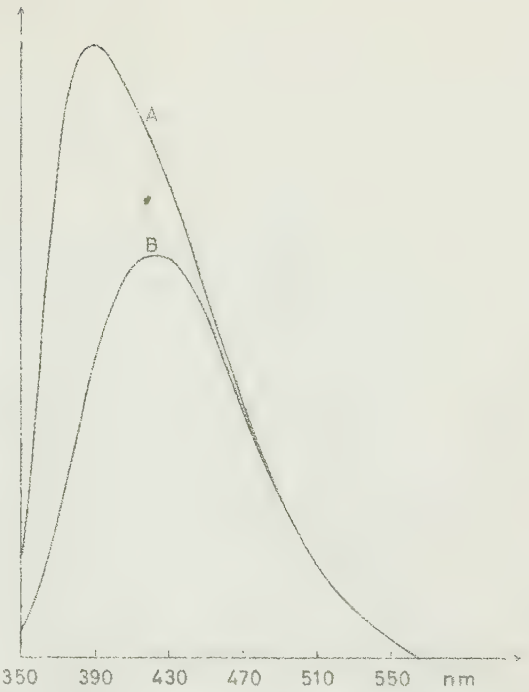

Fig. 1. Relative fluorescence intensity at different wavelengths.
Excitation wave-length 310 nm
Excitation band-width 25 nm
Emission band-width 5 nm
River water from the Nissan below (A) and above (B) paper and pulp mill.

Base materials

Sodium lignin sulfonate A was prepared from sodium bisulfite cook for 2 hours at 170°C. The initial pH was 4 and at the end 3.2. The sulfite and sulfate salts were precipitated with barium chloride. The residue was then purified on Sephadex G10 and 85 % of the lignin sulfonate polymer fraction was isolated.

Sodium lignin sulfonate B was prepared by the Finnish paper and pulp research institute. It was extracted with amine from a technical acidic calcium bisulfite cook. The percentage molecular weight distribution was

< 5000 30.9 %
> 5000 69.1 %
> 10000 53.2 %

and the absorptivity at 280 nm was 13.7 l/g cm.

Björkman-lignin was dissolved in chromatographic purified dioxan and distilled water (1: 1).

Aquatic humic substances were isolated from water from the Göta river with Chelex-100 saturated with iron (III) chloride. After adsorption the humic substances were eluted with 3 M ammonia. It was characterized by following values; 6.79 % N, 1.65 % OCH_3 and 11.72 % ash content.

Copenhagen standard sea water.

Natural samples

Seven samples of different origin were investigated:

1. Sea water from the Byfjord (*Byfjorden,* a firth in the northern part of the Swedish West Coast).
2. River water from the Nissan; upstream a paper and pulp mill (Hylte bruk).
3. River water from the Nissan; 2 km downstream a paper and pulp mill (Hylte bruk).
4—7. Brackish water from the Hanö bay of the Baltic; 4 different stations.

General procedure

In the operation of the instrument, samples were irradiated at selected wave-lengths between 200 and 600 nm by manual adjustment of the excitation monochromator. At every 30 nm intervals emission spectra were automatically scanned from 350 to 500 nm. A base line was established with distilled water or standard sea water and checked repeatedly during the cause of all measurements. The measurements were performed with the excitation band width of 25 nm and emission band width of 5 nm. The calibration curves were, however, measured with an emission band-width of 25 nm.

Results

For the natural waters investigated, the excitation at 310 nm was most suitable for our purpose. The difference in emission intensity at 390 nm between lignin sulfonates and the humic substance is larger for excitation at 210 nm, but the emission intensity is considerably higher for excitation at 310 nm. Direct measurements on river water above and below a paper and pulp mill revealed an increase of fluorescent substances *(Fig. 1).* As it can be seen from this figure the peak is not only strongly amplified, but there is also a shift in the peak wavelength from 425 nm to 390 nm. The down-stream peak has also a definite shoulder. The automatic scan rate was always fast and no change depending on photodecay could be observed upon repeated scanning. Samples from the Hanö bay showed *(Fig. 2)* very small differences in emission intensity, but the sea water sample from the Byfjord has a lower intensity.

The base materials, humic acids, lignin sulfonate A and B and Björkman lignin, give different emission curves as may be seen in *Figs 3* and *4.* The peak wavelengths are 415, 365, 390 and 360 nm respectively. By substracting the upstream curve in Fig. 1 from the downstream curve, a curve with peak at 375 nm is obtained (see *Fig. 5).* This resulting curve lies between the

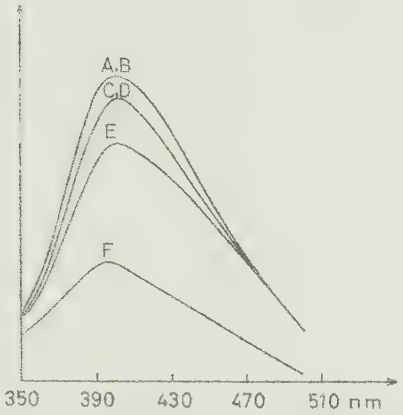

Fig. 2. Relative fluorescence intensity at different wavelengths.
Excitation wave-length 310 nm
Excitation band-width 25 nm
Emission band-width 5 nm
Brackish Baltic water from 4 different stations in the Hanö bay (A, B, C and D), sea water from the Byfjord (E) and Copenhagen standard sea water (F).

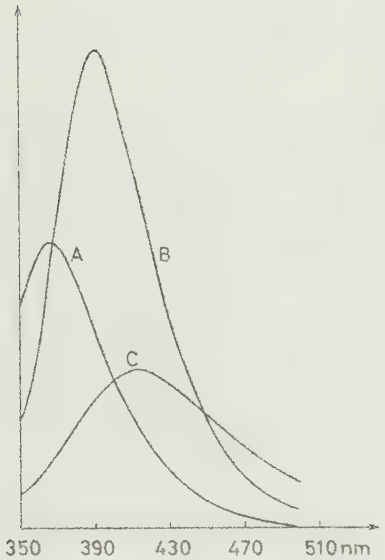

Fig. 3. Relative fluorescence intensity at different wave-lengths.
Excitation wave-length 310 nm
Excitation band-width 25 nm
Emission band-width 5 nm
10 ppm of lignin sulfonates A and B and humic substances (C) in distilled water.

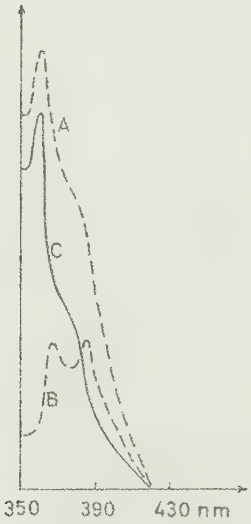

Fig. 4. Relative fluorescence intensity at different wave-lengths.
Excitation wave-length 310 nm
Excitation band-width 25 nm
Emission band-width 5 nm
10 ppm of Björkman lignin in dioxan and water 1: 1 (A), dioxan and water 1: 1 (B) and the intensity difference between them (C).

Fig. 5. Relative fluorescence intensity att different wavelengths.
Excitation wave-length 310 nm
Excitation band-width 25 nm
Emission band-width 5 nm
10 ppm of lignin sulfonate A and B in distilled water and the intensity difference between river water from the Nissan below and above paper and pulp mill (C).

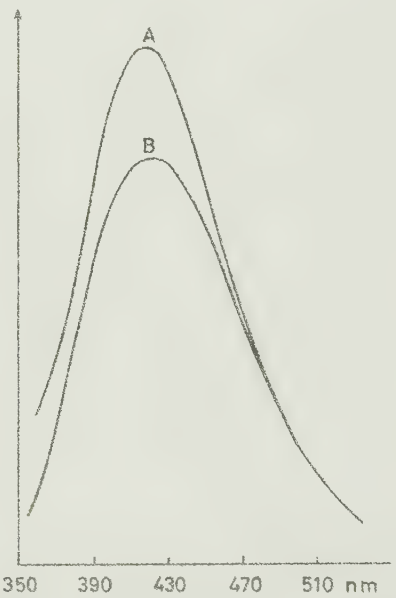

Fig. 6. Relative fluorescence intensity at different wavelengths.
Excitation wave-length 310 nm
Excitation band-width 25 nm
Emission band-width 5 nm
10 ppm of humic substances in distilled water (A) and river water from the Nissan above paper and pulp mill (B).

two types of lignin sulfonates A and B as also may be seen from Fig. 5. *Fig. 6* shows that the humic substance isolated from the Göta river has a maximum at almost the same wavelength as the sample from the river Nissan upstream the paper and pulp mill. In order to evaluate the amount of lignin sulfonate downstream the paper and pulp mill at the river Nissan a standard addition technique was tested. Thereby lignin sulfonate B was added to the upstream river water. The samples were excited at 310 nm and the emission intensity was determined at

390 nm (cf. Fig. 1). A linear calibration curve was found in the range 0-10 ppm lignin sulfonate B. The intensity ratio of (river water + 10 ppm lignin sulfonate B)/(river water) was 69/16, which means that it is possible to measure the addition of approximately 0.2 ppm lignin sulfonate without the use of the "meter multiplier". From this calibration curve the lignin sulfonate content of the river Nissan downstream the paper and pulp mill was calculated to be 3.25 ppm. With the use of the "meter multiplier" a similar calibration was made for excitation at 210 nm, but due to serious inner filter effects the calibration curve was linear only to 5 ppm lignin sulfonate B.

The fluorescence spectra did not shift in the pH range of natural waters (approximately 4 to 8.2).

From *Fig. 7.* it may be seen that the UV absorption spectra at pH 7 of the two lignin sulfonates A and B are rather different from the spectrum of the isolated humic substances. The absorptivity per weight unit is larger for the humic substances in the whole wavelength range of 250 to 360 nm. Thus, normally the UV absorption of lignin sulfonates will be concealed by the absorption of humic substances in Swedish natural waters.

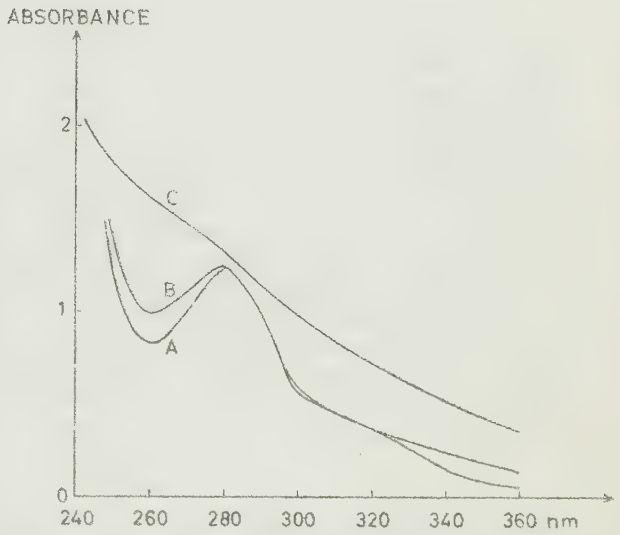

Fig. 7. UV absorption spectra for 10 ppm of lignin sulfonates A and B and humic substances (C) in distilled water.

Discussion

Lignin sulfonates

There are definite differences in several physical and chemical properties between lignins produced by kraft, sulfite, bisulfite, neutral sulfite and soda pulp mills. The nature of lignin by-products will therefore depend on the pulping process itself. Thus, the two lignin sulfonates have different absorptivities at the 260 nm minimum (see UV spectra in Fig. 7). Furthermore, the emission peak maxima are quite different (Fig. 3). The isolation procedure might have contributed to these differences, but the results indicate that it is advisable to obtain a reference sample from the potential source of introduction when investigating the degree of lignin sulfonate contamination of a natural water.

Humic substances

Naturally occurring compounds such as humic substances will interfere in the determination of lignin sulfonates by the fluorescence method as shown in Figs. 1 and 3. Polyphenolic compounds to which the humic substances belong are widely distributed in natural waters. They are best described as a series of acidic, yellow to black colored moderately high-molecular-weight polymers which have characteristics dissimilar to any of the organic compounds occurring in living organisms. Humic substances represent a very heterogenous mixture of molecules and the water soluble fulvic acids isolated for this work may not be quite representative for humic substances in general from widely different localities (cf. Ogner and Schnitzer (13). Waksman (14) proposed that the humic substances represent modified lignins because they have properties similar to oxidized lignins. It is therefore quite natural that humic substances and lignin sulfonates have similar fluorescence spectra. Very little is known, however, about chemical structure of humic substances, their origin, synthesis, degradations and reactions. Even when lignin is a principal organic material being humified, the known chemical nature of humus leads one to believe that other chemical moities must react with lignin degradation products before a true humus will appear (15, 16, 17). The main functional group in humic acids is the carboxylic group and methoxyl content is generally very low ($< 1\ \%$). Lignin has almost no nitrogen in contrast to humic acids (18). However, both may be regarded as polyphenols. Amino acids are present as linking molecules between quinoid structures in humic acids; to a less extent they are bound by ionic forces or by chemisorption. Algae lichcens and mosses which do not contain lignin give rise to humic substances too (cf. Eglinton and Murphy (19)).

Flaig (20) proposed the following sequence for the natural synthesis of humic acids:

1. Lignin, freed of its linkage with cellulose during the decomposition of plant residues, is subjected to oxidative splitting with the formation of primary structural units.
2. The side chains of these units are oxidized, demethylation occurs, and the resulting polyphenols are converted to quinones by polyphenoloxidases.
3. Quinones arising from lignin, together with those synthesized by microorganisms, react with nitrogenous compounds to form dark-colored polymers.

The conversion process of lignin forming humic acids is a rather complicated heterogeneous system since lignin is hardly soluble in water. When the effluent of sulfite paper and pulp industries interact with sea water, a coagulation of lignin sulfonates takes place resulting in precipitation. Furthermore, the lignin sulfonates are subjected to microbial decomposition. The time for conversion is of importance for tracing and determining lignin

sulfonates. Kalninsh et al. (21) describe different processes for removal of lignin sulfonates in sea water, where besides coagulation even bacterial oxidation and sorption of lignin sulfonates occur on sedimenting particles. The conversion processes are in general rather slow and measurements can be made quite a long distance from the source.

Sensitivity of fluorescence measurements

The intensity of the fluorescence observed is proportional to the product of concentration and intensity of the exciting light. Since with modern photomultipliers exceedingly low intensities of light can be measured, it should be possible to detect lignin and humic substances in the concentration range of 0.2 ppb with an advanced spectrofluorimeter. The detection of small amounts of lignin sulfonates in the presence of ppm quantities of humic substances is, however, much less favorable as shown above. With the use of the "meter multiplier" it might be possible to detect approximately 20 ppb of lignin sulfonates in natural waters.

Apparatus

As the apparatus used in these measurements is one of the most advanced on the market it is not realistic to apply it for routine monitoring field control. We used broad half band-widths for the analysis with the intention to make results applicable to a filter fluorimeter of simpler construction like the Turner Model 110 used by Christman (8). A knowledge of the absorbance and the fluorescence spectra of the desired components is essential for selecting the proper filters. The results from one apparatus to another are not easily translated directly, but the fluorescence of lignin sulfonates should be measured in the range of 370—390 nm. We propose a simple cheap home-made filter fluorimeter consisting of a stabilized mercury lamp, appropriate narrow-band interference filters and photomultiplier. The quartz cell should be of the continuous flow type with some kind of pumping system for the polluted water. The signal from the photomultiplier can be recorded continuously and from time to time a reference standard solution should be employed.

Acknowledgements

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IV

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A simple apparatus for on site continuoús liquid-liquid extraction of organic compounds from natural waters

Martin Ahnoff and Björn Josefsson

Department of Analytical Chemistry, University of Gothenburg, Fack, S-402 20 Göteborg 5, Sweden.

A considerable number of methods (cf. Goldberg (1)) have been worked out to detect qualitatively and quantitatively the presence of organic compounds in natural waters. Direct measurements and identification of organic compounds in water are complicated because of sensitivity and specificity requirements. Polluted water contain many and varied natural and synthetic compounds that would seriously interfere with subsequent identification and quantification.

The amount of organic substances especially pesticides in natural waters is usually very small. Nowadays the separation and identification techniques are highly sophisticated when employing gas chromatography and mass spectrometry, which can produce information for identification at high sensitivity especially when using on-line data acquisition systems. Even so in trace analysis the concentration step from a large mass of water will further increase the sensitivity.

Activated carbon filter has been employed for adsorption of different kinds of organics in natural waters since it was developed and introduced by the U.S. Public Health Service (2).

However, the lack of adsorption and desorption control in addition to bacterial and oxidizing attack on the organics limits the method (3). Better results both in extraction and recovery from filter materials were found with reversed liquid--liquid partition using a hydrophobized carrier coated with a lipophilic stationary phase followed by recovery from the column by means of small amounts of organic solvents. Ahling and Jensen (4) used Carbowax 4000 monostearate and n-undecan as stationary phase when analyzing pesticides. Waters with high particle content were first treated with aluminum sulfate to remove the particles before the filtering process. Aue et al.(5) used silicones which were chemically bonded to diatomaceous earth particles to extract non-polar organics from water. Ito (6) tested covalently bonded stationary phases of aromatic and alkyl chlorosilanes on celite. His results were not satisfactory for concentrating pesticides from a large amount of water. Gesser et al. (7) published the use of porous polyurethane foam columns for extraction of polychlorinated biphenyls (PCB) compounds from water. Uthe et al. (8) found that uncoated porous polyurethane plugs gave poor recovery of pesticides. Therefore they coated the polyurethane foam with selective adsorbents and got better recovery.

Burnham et al. (9) were enriching many neutral organic compunds from potable water using macroreticular resins. They used columns filled with Amberlite XAD-2 and XAD-7 resin (Rohm and Haas). Dissolved organics in sea water were concentrated by Riley and Taylor (10) using a column packed with Amberlite XAD-1 the first manufactured macroreticular adsorbent in the XAD series. Ahling and Jensen (4) reported that the Amberlite XAD resins were efficient for adsorbing chlorinated pesticides,

however, reextraction from the filter material gave poor recovery

An alternative to filtering processes is batch or continuous solvent extraction. Since a large mass of water is to be extracted the batch methods are limited although Kawahara et al. (11) constructed a semiautomatic device to speed up the procedure and reduce the organic solvent volume. Batch methods are usually constructed without continuous refreshing systems. Instead a repeated shaking with a fresh portion of solvent is used. Schafer et al. (12) used a batch method to determine pesticides in water where a 3.5 liters sample was stirred to obtain a vortex mixing with 10 ml of hexane at a ratio of 1:350. Standard Methods of Water and Wastewater 1971 (13) recommend this semiautomatic L.L.E. method with two serial extractions with 10 ml of hexane for chlorinated hydrocarbons. During the investigation of pollution in coastal sea waters Werner and Waldichuk (14) pointed out the need for concentrating and isolating trace amounts of certain substances with a continuous solvent extractor. They constructed a modified Scheibel (15) apparatus by changing the organic solvent cycle system. Kahn and Wayman (16) reported a continuous multichamber liquid-liquid extractor with internal solvent refreshing for the extraction of nonpolar contaminants especially pesticides from natural waters. With this apparatus it was possible to extract extremely large water samples at rates of 0.5 to 1.0 liters per hour, and in that way isolate and identify very minute concentrations of pesticides with a recovery of 97 per cent. Goldberg et al. (17) presented a similar apparatus which was capable of handling flow rates up to 2 liters per hour and designed to operate with heavier-than--water solvents. In a later work Goldberg et al. (18) described

modified extractors for both heavier-than-water and lighter--than-water solvents. Three solvent heavier-than-water extractors were set up in one series and four solvent lighter-than-water extractors were set up in a second series. Spiked water was pumped at a rate of 7 to 8 liters per hour through each set of extractors and the extraction efficiencies were determined. A concentration factor of up to 10^5 was obtained and the dipole moment difference between the solute and solvent was demonstrated to be an index of the extraction efficiency.

Our experience with various filtering materials has confirmed the difficulties with such materials although filter columns are simple to handle at field. Particles, which adsorb pesticides, will either run through the adsorbtion filter or reduce the flow when stopped. A pre-filter will remove the particles, but may change the initial composition of organics in the water. This favours liquid-liquid extraction.

The apparatus of Kahn and Wayman (16) was changed by the authors to fit field conditions by using one chamber and enlarging the outlet tube. The phase separation became more efficient and the stirring rate could be increased without loosing drops of the organic solvent. This arrangement is similar to that described by Kerner et al. (19) who used a bulb for better phase separation. However, a system where the organic solvent is recycled is too complicated for routine field application especially when immersed in the water. The changes of the outer temperature will disturb the heating of the solvent. The few theoretical plates in the column between the evaporation flask and the reflux condenser do not prevent the organic compounds to cycle together with the solvent.

Water is dissolved in the organic solvent and remains in the vaporizing flask. The boiling of the solvent together with water causes e.g. uncontrollable heat changes, hydrolysis reactions and steam distillation. The question arised whether it was possible to exclude the solvent refreshing system. From this starting-point a new construction was worked out to fulfil the requirement of simplicity and efficiency.

Experimental

Apparatus. The apparatus constructed by the authors (Patent pending) for continuous extraction of water with a stationary lighter-than-water solvent is shown in Figures 1 and 2. In principle it is a mixed settler although the mixing and settling chambers are not completely separated but combined in one cylinder. The water is continuously drawn through the cylinder under vigorous mixing with a ligther-than-water organic solvent, thereby forming a vortex. The mixing is performed by a magnetic stirrer (A) located under the cylinder. The organic solvent is trapped by a flange in the cylinder and is not replenished during the procedure. The mixing of the water and solvent takes place in the upper part of the cylinder and the emulsion is separated successively in the lower part. The lighter solvent droplets are allowed to rise and return into the mixing zone. In this way very little of extracting solvents is lost during the procedure. It is recommended to use a solvent volume of 100-300 ml with the 800 ml apparatus shown in Figure 1.

The apparatus consists of three main parts: the extraction unit, the magnetic stirring device (A) (Metrohm, E349, Switzerland) and the pump (B) (ProMinent Electronic 0304T, CFG, W-Germany).

The extraction unit is designed in four parts: top (C), bottom (D), bowl (E) (all in PTFE) and a Pyrex glass cylinder (F), 80 mm (i.d.) by 190 mm, and a wall thickness of 5 mm. The top and bottom parts are pressed water tight to the glass cylinder by plates of acid-proof stainless steel (G) and four screwbolts (H). The bottom steel plate has an opening in the middle (65 mm in diameter) to actuate the stirring bar inside (I). In the top part are two channels (3 mm in diameter), one for the water inlet and one, equipped with a stopcock, for the filling and the draining of the apparatus. The bottom part (D) has a circular hollow to fit the bottom of the bowl (E). Three symmetrically placed holes (K, Figure 2) at the edge of the hollow are in contact with a circular channel (L) which leads to the outlet tube (B). In this way the extracted water is sucked out from the calm circular compartment (O) with a symmetrical flow. The bowl (E) is designed to keep the mixing process above and inside whereas the outside of the bowl acts to separate the emulsion and return the organic solvent into the mixing zone. The bowl has a double flange (M) to quell the vertical emulsion movement. The circular cross--section area between the flange and the glass wall is important because the flow rate acts to bring the emulsion downwards while the lighter solvent is striving upwards. The rotating movement in the upper compartment is definitely quelled at N (Figure 1 and 2) by letting the water pass 36 holes (3.6 mm in diameter). The final phase separation occurs in the cavity (O) below the holes where the mixing procedure has no influence. By this arrangement of the settling zones most of the lighter solvent is trapped and returns to the mixing compartment provided the flow rate is not too high

(see below). Since there is no pressure drop in the apparatus, the pump can be placed at the outlet. Thus contamination caused by the pump is eliminated.

Materials. The cyclohexane used as extracting agent was purified by distilling technical grade cyclohexane on a bubble cap plate column with 12 plates, using a reflux ratio of 12:1. The purity was checked by concentrating 200 ml of the solvent to 1 ml and injecting 10 microliters into the gas chromatograph. Cyclohexane nanograde (Mallinckrodt) also proved to be sufficiently pure, and was used in some experiments. n-Hexane min. 85% (Merck) was distilled and checked in the same way as described for cyclohexane. The standard pesticide solution used in the laboratory extraction tests contained 5 µg Lindane, 10 µg Aldrin , 25 µg Dieldrin , 20 µg p,p′-DDE, 40 µg p,p′-DDD and 60 µg p,p′-DDT (all 99.9% pure) per ml of acetone AR (Merck). Clophen A-60 (Bayer), a mixture of polychlorinated biphenyls (PCB) with 60% chlorine content, was used as a gas chromatographic standard for the PCB determination in the river water extracts. In the clean up procedure employed for the PCB analysis, Florisil 60-100 mesh (Matheson Coleman & Bell) and sodium sulfate AR (Merck), were used. Both reagents were heated to 600°C in 4 hours and kept at 130°C in glass stoppered vials.

Laboratory Test Procedure. Three extractors were set up in one series connected by PTFE tubing. Each extractor was charged with 150 ml cyclohexane and filled up with triple distilled water. In a 10 liter glass flask, which serves as a feeding reservoar, 8 liters of triple distilled water and 1 ml of standard pesticide solution were introduced 10 minutes before the extraction started. A magnetic stirrer mixed the spiked water throughout the whole

experiment. The outlet of the third extractor was connected to a pump which sucked the water from the feeding reservoir through the three extractors. The effluent water was collected in another 10 liter glass flask. After the 8 liters had been sucked through the system, one liter of triple distilled water was added twice to the feeding reservoir and pumped through the system, the extractors still being in action. This was made to ensure that all of the spiked water had passed through all three extractors.

After the extraction the three extractors were turned upside down and drained. The extracts were examined on the gas chromatograph. The feeding reservoir, the drained first extractor and the effluent water were shaken vigorously with 100 ml of cyclohexane. These samples were also analysed. The standard pesticide solution used to spike the water was also used to prepare the gas chromatographic standard. Thus the amounts of pesticides found in the different samples were determined as fractions of the amount added to the feeding reservoir.

Field Procedure. The extractors were placed on the river bank near the water surface. The pump sucked the river water unfiltered through the extractors. They were arranged to extract in a series and parallel extractor trains. Before use, the extractors had been throughly cleaned. To check the background of interfering contaminants deriving from the extractor as well as the clean up procedure, a blank was prepared from each extractor. The extractors were filled with 250 ml cyclohexane and triple distilled water and the content was stirred vigorously for 24 hours. The cyclohexane was then treated in the same way as the river water extracts.

Treatment of Extract before Gas Chromatography. The extracts from the test procedure were injected into the gas chromatograph without previous treatment. The extracts from the field procedure were concentrated to 5 ml in a Kuderna-Danish concentrator and a tube heater (Kontes, K 720000). The concentrate was placed on a 10 cm Florisil column (2 cm i.d.) covered with 2 cm of sodium sulfate, and eluted with 200 ml of hexane. The column had been prewashed with 100 ml of hexane. The elute was concentrated to 1 ml in the same apparatus as above, and 10 µl portions were injected into the gas chromtograph.

Gas Chromatography. A Hewlett-Packard Model 7620A dual column gas chromatograph equipped with an electron capture detector (200 m Ci tritium; pulsed system) was employed. Glass columns (2 m x 2 mm i.d.), silanized with dimethyldichlorsilane, were used. The solid support was 80/100 mesh Chromosorb W DMCS coated with 3% OV-1, 3% QF-1 and 3% OV-17/3% XE-60 (1:1) respectively.

The conditions for the chromatography were: injector 230°C; column 195°C; detector 215°C. Helium was used as the carrier gas at a flow rate of 40 ml/min. The purge gas was argon with 10 per cent methane using a flow rate of 60 ml/min. The electron capture detector pulse interval was 50 µs and the standing current on the chromatograph was held steady during the analysis at 5×10^{-9} A. The peak height was used for the quantitative evaluation. Dieldrin and p,p'-DDE were not separated on the OV-1 column; accordingly the sum of them is reported. The quantitative calculation of the PCB was made on five characteristic matching peaks in the sample and the standard. The sum of the peak heights in the sample and the standard was calculated and compared.

Results.

Laboratory Test. Tables I and II show the fractions of the different pesticides found in the extractors as well as the feeding and collecting reservoars, for pumprates of 2 and 5 liters per hour respectively. The values are expressed in per cent of the amount introduced in the feeding reservoir. The total recovery ranged between 95.1 and 102.4 per cent except for Aldrin which gave lower recovery. Most likely, the loss of Aldrin is due to its poor stability when exposed to water and air.

The amount of pesticides adsorbed on the glass walls in the feeding reservoir was considerable and showed a marked increase with contact time, see Table IV. Table III shows calculated values of the extraction efficiency in the first extraction step for pumprates of 1.1, 2 and 5 l/h. At a pump rate of 2 l/h, the extraction efficiency was 89-96 per cent. As the pump rate increases to 5 l/h, the extraction efficiency decreases to 83-86 per cent. Consequently, larger fractions are found in the second and third extraction steps in this case (Table II).

Field Experiments. Table V presents the amounts of polychlorinated biphenyls found in the river water extracts from different field experiments at the Göta river. Pesticides in the Göta river water were below our detection limit. Three different extraction arrangements were set up in five experiments: two parallel extractor experiments, and three experiments with the extractors arranged serially. The waterflow varied as well as the total amount of water pumped through the system.

Figure 3 shows the gas chromatogram from OV-1 column of extract from the Göta river water on 73-01-31 and a Clophen A-60 standard. The two chromatograms are not identical but at least 11 Clophen peaks are present in the river water extract. Confirmatory studies were carried out on three different columns.

Discussion.

Theory. A theoretical model for continous extraction of water with a stationary organic phase was worked out with the following presumptions:

1) The compound, which is to be extracted, is distributed between the two phases so that at equilibrium
$$\frac{[\text{concentration in organic phase}]}{[\text{concentration in aqueous phase}]} = K$$
where K is the distribution constant.

2) The water that has passed through the extractor is in equilibrium with the organic phase with respect to the compound to be extracted.

3) The concentration of the compound in the incoming water, C_{aq}, is constant. V_{org} is the volume of the organic phase and is constant. C_{org} is the concentration in the organic phase and is zero when the extraction starts at $V_{aq} = 0$.

The extraction process can then be described by:

$$C_{org} = C_{aq}K(1 - e^{-V_{aq}/V_{org}K}) \qquad (1)$$

For high values of K and low values of C_{aq}, equation (1) takes the form:

$$C_{org} \approx C_{aq}V_{aq}/V_{org} \qquad (2)$$

which means that the extraction efficiency is close to 100%.

For low values of K and high values of V_{aq}, equation (1) reduces to:

$$C_{org} \approx C_{aq}K = \text{constant} \qquad (3)$$

which shows that the concentration in the organic phase has reached its upper limit, determined by the distribution constant K and the concentration in the water C_{aq}.

To show what this means in practice, extraction curves have been calculated for some pesticides with given K values (20). Assuming that V_{org} = 0.250 (liter), the maximum water volume giving an extraction efficiency of at least 95%, can be calculated. This is illustrated in Figure 4.

According to this model, the theoretical extraction efficiency for the first extractor in the laboratory extraction test should be nearly 100%, for all components extracted. This is, approximately, the case with a pump rate of 2 l/h. With a pump rate of 5 l/h this is clearly not the case, indicating that the contact time was too short to permit equilibrium to be reached between the effluent water and the organic phase.

With this result in mind, the extraction efficiency for polychlorinated biphenyls in the field test might be expected to lie between 85 and 100%. The K values of PCB:s are not known, but since they are at least as high as K for DDT, it should be possible to extract hundreds and even thousands of liter without loosing in extraction efficiency. However, the serial extraction experiments show high amounts of PCB also in the second extraction step. The explanation to this anomaly is probably that the PCB do not exist in true solution.

Sorption Effects. Hill and McCarty (21) indicated that sorption of pesticides on algae may be of considerable magnitude. Aquatic microorganisms absorb and concentrate pesticides from water apparently inversely related to the water solubility of the compound (22). Sorption of PCB on particles as well as interaction with neutral organic material (23) may explain the state of PCB in river waters. Therefore, the PCB:s may not be extracted in the same way as the pesticides in the laboratory tests. Another explanation is that the presence of fulvic acids may solubilize PCB in water to such a degree that the operational distribution constant K (cyclohexane to water) is too low to permit full recovery of PCB. A recovery of 60 per cent in the first extractor (see Table V) roughly corresponds to a K-value of 10^3.

Choice of Solvent. The choice of solvent is restricted by the requirement of low solubility in water. Both hexane and cyclohexane fulfil this condition. However, with cyclohexane better phase separation is obtained, which means lower loss of solvent in the form of small droplets. This is probably explained by differences in viscosity and surface tension. The loss of solvent is affected by water quality parameters such as turbidity and salinity. Therefore the loss of solvent must be determined in the actual natural water. Experiments with the Göta river water have shown that 25 ml of cyclohexane was lost by dissolution and 25 ml as droplets when extracting 200 l of water. Knowing this, the results can be compensated for solvent losses.

Acknowledgement.

The authors wish to thank David Dyrssen for valuable discussions. We are also indebted to Sören Jensen for the provision of the polychlorinated biphenyls and to Birgitta Jarmark for technical assistance in the PCB analysis. The work on the analytical chemistry of natural waters at our department has been supported by Göta älvs Vattenvårdsförbund (Göta River Water Protection Federation) and the Swedish Natural Science Research Council.

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Abstract

A continuous liquid-liquid extraction apparatus based on mixed settling is described. Simple and robust performance made it possible to use in field conditions. The organic refreshing system, normally used, is eliminated. The theory for extracting chlorinated pesticides continuously from water with a stationary immiscible solvent is discussed. In laboratory tests the recovery of added pesticides is 83 - 96 % for different pesticides and different pump rates. Polychlorinated biphenyls (PCB) were extracted from some hundred liters of water at the Göta river with different multichamber arrangements. Finally the content of PCB was determined with ECD gas chromatography in the range 0.1 - 1.0 ng per liter water.

Brief

A continuous solvent extractor, designed for the extraction of organic trace substances at field, is described. Polychlorinated biphenyls (PCB) were determined in river waters at sub ppt range.

Table I. Fractions of pesticide standard found at a pump rate of 2 l/h .(%).

	Lindane	Aldrin	DDE + Dieldrin	DDD	DDT
Feeding reservoir	0	5.1	3.6	3.0	5.2
First extract	94.8	78.3	87.6	91.0	82.9
Drained first extractor	1.2	1.2	1.4	1.4	1.5
Second extract	2.8	3.6	3.5	3.9	3.5
Third extract	0	1.8	1.3	1.6	1.9
Effluent water	0	0.3	0.2	0.1	0.2
Total	98.7	90.3	97.5	101.0	95.1

Table II. Fractions of pesticide standard found at a pump rate of 5 l/h. (%).

	Lindane	Aldrin	DDE + Dieldrin	DDD	DDT
Feeding reservoir	0	3.7	2.9	1.2	4.6
First Extract	81.0	69.4	79.2	83.2	79.1
Drained first extractor	2.0	1.8	2.0	2.0	2.0
Second extract	13.2	9.5	10.5	11.1	9.7
Third extract	2.4	3.6	3.9	4.2	4.1
Effluent water	0	0.7	0.5	0.7	0.7
Total	98.7	88.7	98.9	102.4	100.3

Table III. Extracing efficiencies in the first extractor at different pump rates. Correction is made for adsorption in the feeding reservoir.

Pump rate liter/hour	Lindane	Aldrin	DDE + Dieldrin	DDD	DDT
1.1	93	78	92	96	89
2.0	96	84	92	95	89
5.0	83	74	84	86	85

Table IV. Fractions of pesticide standard adsorbed on glass walls in feeding reservoir at different contact times.

Mean contact time, hours.	Lindane	Aldrin	DDE + Dieldrin	DDD	DDT
1.0	0	3.7	2.9	1.2	4.6
2.5	0	5.0	3.4	2.9	4.9
4.5	0	15.8	14.2	11.3	24.2

Table V. Concentration of PCB found in Göta river water at ca 1 m.

Arrangement	Date	Water volume liters	pump rate liter/hour	PCB ng/liter
parallel	72 09 08	290	5.0	0.31
	72 09 08	305	5.2	0.29
parallel	73 01 17	170	3.5	0.21
	73 01 17	185	3.8	0.22
series 1				
extractor 1	73 01 29	190	3.8	0.32
extractor 2	73 01 29	190	3.8	0.16
series 2				
extractor 1	73 01 31	170	3.4	0.20
extractor 2	73 01 31	170	3.4	0.12
series 3				
extractor 1	73 06 03	240	3.0	0.28
extractor 2	73 06 03	240	3.0	0.17
extractor 3	73 06 03	240	3.0	0.09

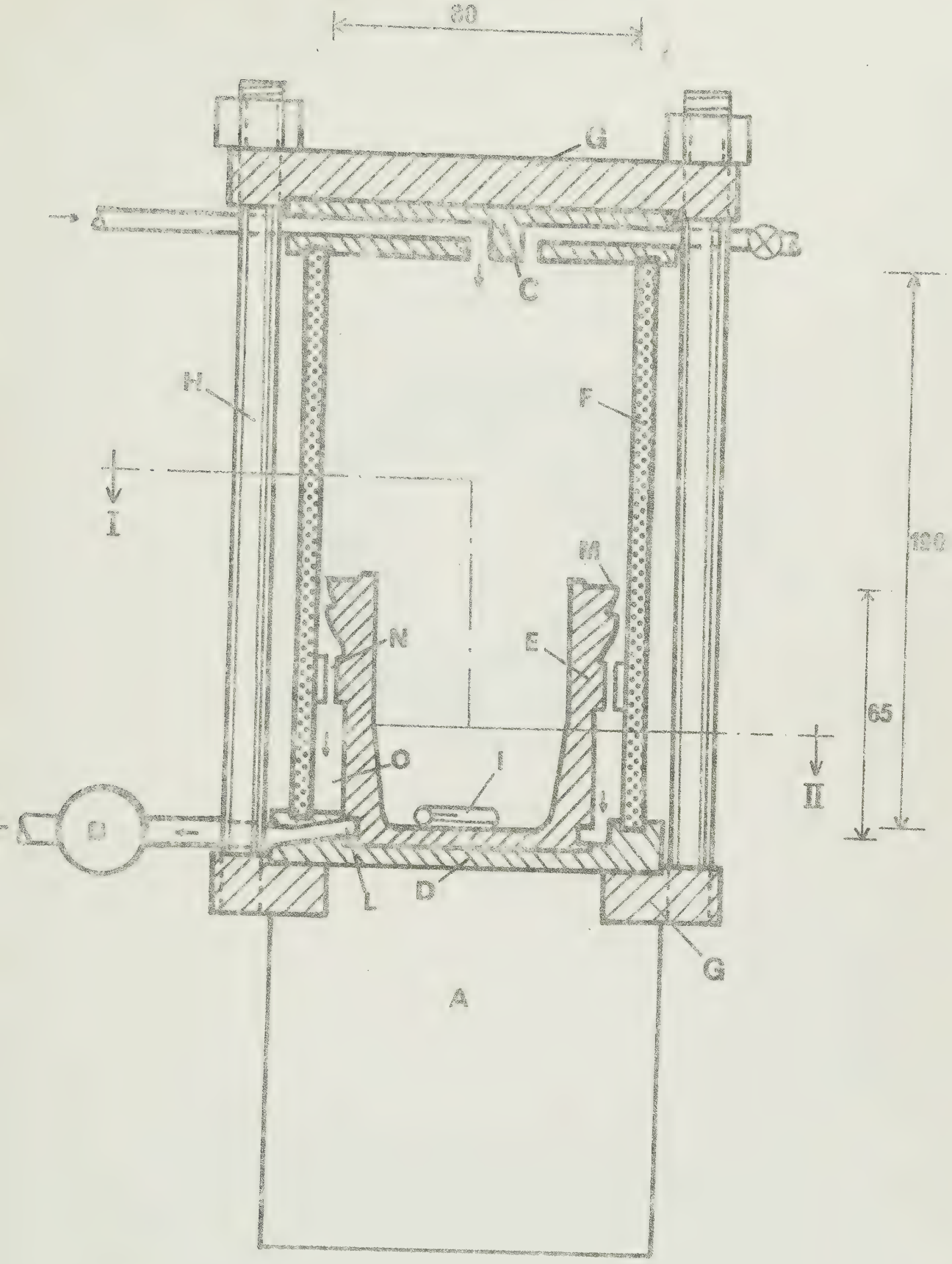

Figure 1. Schematic diagram of the mixed settler (dimensions given in mm).

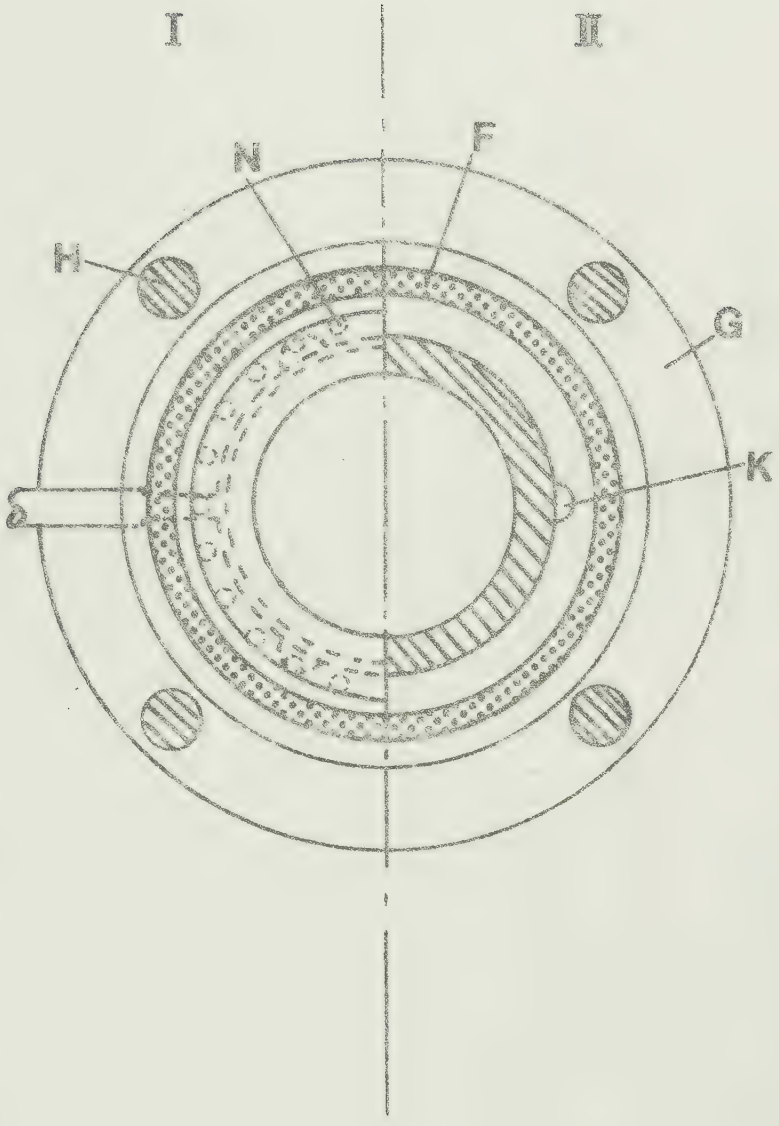

Figure 2. Cross section at I and II from the apparatus shown in Figure 1.

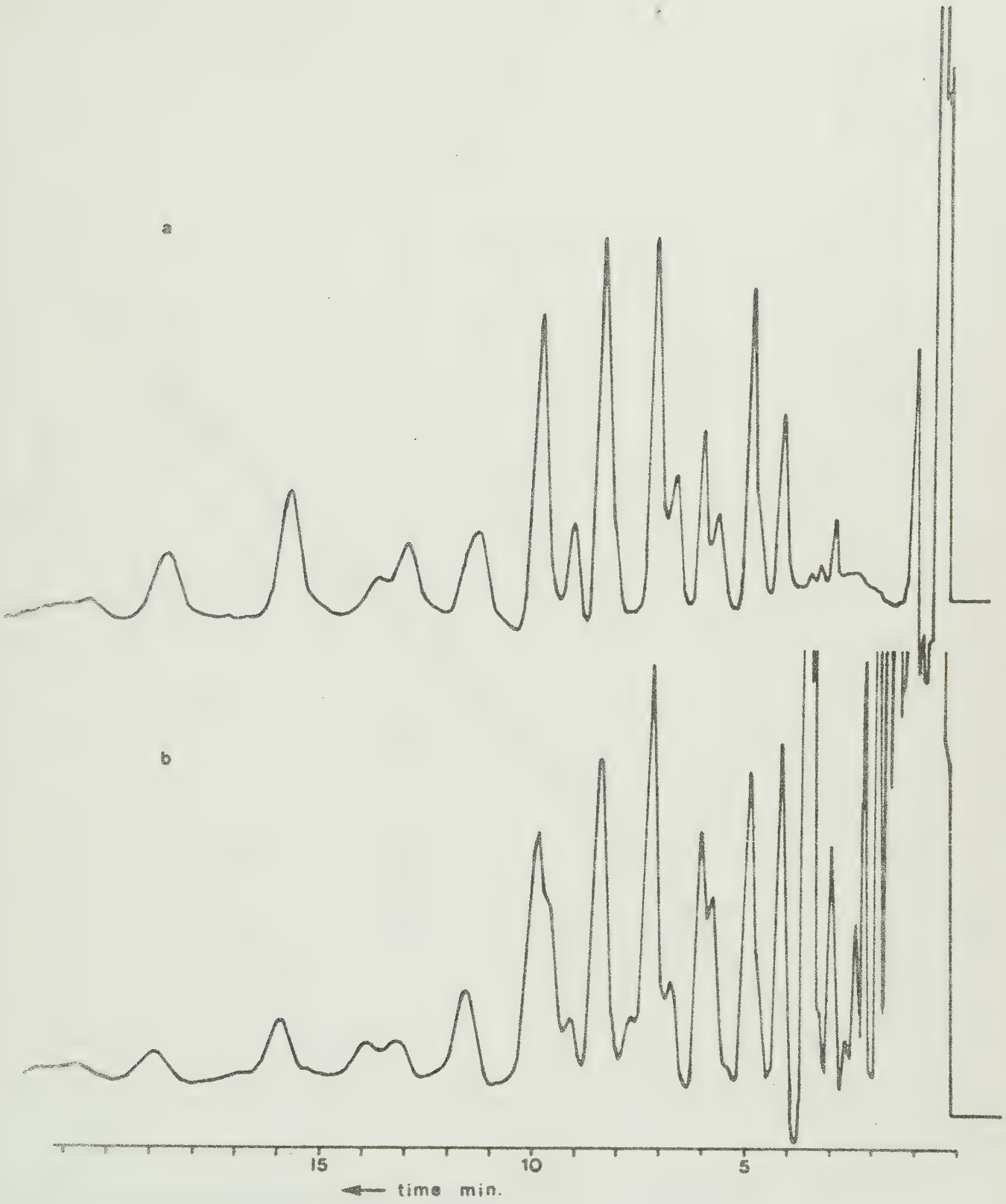

Figure 3. Gas chromatograms of (a) polychlorinated biphenyls standard, 0.5 ng Clophen A-60 injected and (b) extract from Göta river water.

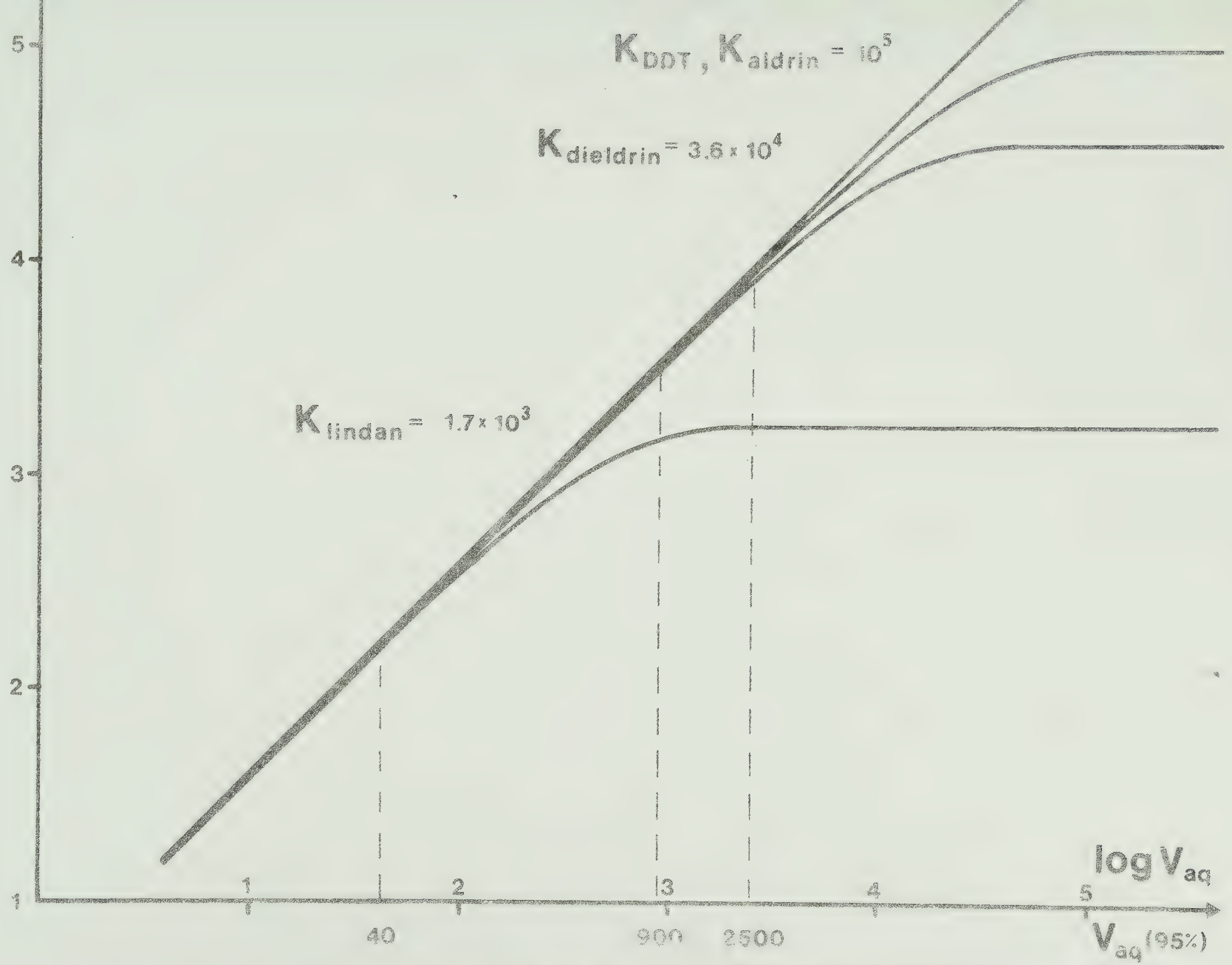

Figure 4. The extraction curves for Lindan, Dieldrin, Aldrin and DDT [illegible] showing that

CONFIRMATION STUDIES ON POLYCHLORINATED BIPHENYLS (PCB) FROM RIVER WATERS USING MASS FRAGMENTOGRAPHY

KEY WORDS : mass fragmentography; multiple ion detection; confirmation; PCB; chlorinated biphenyls; river water

Martin Ahnoff and Björn Josefsson

Department of Analytical Chemistry,
University of Gothenburg,
Fack, S-402 20 Göteborg 5, Sweden

ABSTRACT

A specific and sensitive method to confirm the presence of polychlorinated biphenyls (PCB) in river water is described. Mass fragmentography is shown to be a proper technique especially when utilizing naturally occurring stable isotopes of chlorine. Synthesized tetrachloro-, pentachloro- and hexachloro biphenyls were used to focus the mass spectrometer at three selected masses so called multiple ion detection (MID).

INTRODUCTION

The most widely accepted procedure for confirmation of polychlorinated biphenyls (PCB) in crude extracts consists of cleanup procedure combined with selective gas chromatographic separation followed by relatively specific and sensitive detection. The clean up procedures are mainly column chromatography with Florisil, silica gel and alumina or thin layer chromatography. These techniques are intended to remove the major interfering coextracted materials such as oils, fats and pigments. The next step is the application of gas liquid

chromatography for separation and identification of the PCB extracted from aqueous environments.

The most popular detector utilized is the electron capture detector which is very sensitive to electron capturing substances such as PCB. However, the limited specificity of the electron capture detector is often a source of problems in detecting PCB in extracts from water. A simple gas chromatographic separation does not provide unequivocal identification. Therefore retention time and peak geometry must be compared on two or more different columns. Still, this may not be enough to confirm the structural identity and because the polychlorinated biphenyls are a highly complex mixture of up to 60-70 identified isomers[1] a further confirmation should be employed using infrared or conventional mass spectroscopy whenever the quantity of the compounds permit. If it is not possible other selective detectors have been recommended such as the microcoulometer detector which is more specific than electron capture detectors but less sensitive for field samples containing interfering substances.[2]

An advantage of the mass spectrometric approach is the availability of combined gas chromatograph - mass spectrometer instruments which permit high sensitivity analyses of multicomponent mixtures without previous isolation of pure samples. Because of the definite structural information obtained by mass spectrometry, proper use of the technique would eliminate other confirmation techniques. By using this combination PCB have been found in bald eagles[3], and human adipose tissue[4], fish and birds.[5] Garrison et al.[6] determined PCB in sediment and water extracts. First the presence of PCB was tentatively proved by electron capture gas chromatography. Then they were confirmed by combined

gas chromatography - mass spectrometer. The individual mass spectra were taken on flame ionization detector response peaks and appeared not to be a result of single components. To eliminate the background caused by overlapping or underlaying sample components and column bleed, they took spectra just before and after the peak eluates and subtracted them from the raw spectrum to get the spectrum of the individual PCB. This manual data reduction can be transferred to computers.

Hites and Bieman[7] designed a gas chromatograph - mass spectrometer system which uses a computer to continuously and automatically record spectra of the gas chromatographic effluent. Certain ions or fragmentation patterns of interest could by data reduction then be plotted as a function of the scan number giving so called mass chromatograms. A similar technique was used by Bonelli[8] with a quadrupol mass spectrometer for analysis of PCB in sewer extracts. The isotopic cluster region from chlorine compounds were plotted as a function of the scan number to give a kind of reconstructed gas chromatogram. These computerized data acquisition systems have detection levels in the nanogram range when using the limited mass mode of operation.

Another approach using mass spectrometer as a gas chromatographic detector is mass fragmentography. This technique was introduced by Sweeley et al.[9] and is reviewed by Gordon and Frigerio.[10] The application of mass fragmentography was suggested by Hammar et al.[11] to trace picogram (injected amounts) quantities of drugs in various extracts. Mass fragmentography is the simultaneous monitoring of one or more fragment ions when keeping the magnetic field constant and varying the applied voltage to focus the different ions. The technique differs from conventional mass spectrometry by eliminating the scanning of the total ion

spectrum. The proper selection of masses is crucial in both mass fragmentography and computer evaluation of continuously scanned mass spectra. This is done beforehand with mass fragmentography and afterwards with the other technique. Mass fragmentography is more sensitive than the repetitive scan technique because of a higher signal-to-noise ratio.[7] The sensitivity of detection can be increased by a factor of 1000 to 10 000 over that of conventional gas chromatographic methods with electron capture and flame ionization detectors[10] since the signals are amplified by an electron multiplier. The combination of high sensitivity and high selectivity make mass fragmentography an ideal technique for confirmation studies of extremely low quantities of PCB in river water extracts.

EXPERIMENTAL

Materials. Cyclohexane nanograde (Mallincrodt) was used as extracting agent for the river waters. The n-hexane used was purified by distilling technical grade n-hexane on a bubble cap plate column with 12 plates using a reflux ratio of 12:1. Florisil 60-100 mesh (Matheson Coleman & Bell) and sodium sulfate AR (Merck) used in the clean up procedure were heated to 500°C for 4 hours and stored at 130°C in glass stoppered flasks. Clophen A-60 (Bayer) and Aroclor 1254 (Monsanto) standard solutions were prepared at concentrations of 100 ng/ml and 1 µg/ml in n-hexane. A set of standard solutions of 2,3',4',5-tetrachlorobiphenyl, 2,3',4,4',5-pentachlorobiphenyl and 2,2',4,4'5,5'-hexachlorobiphenyl were prepared in concentrations of 10 µg/ml in n-hexane. These compounds were synthesized by Sundström and Wachtmeister.[12]

Extraction procedure and clean up. The extraction procedure was carried out on site with a continuous liquid-liquid extractor

using 250 ml cyclohexane for extracting 160 liters of unfiltered river waters.[13] The river water was sucked through the apparatus at a rate of 3 l/hr. The cyclohexane phase was concentrated to 5 ml in a Kuderna-Danish evaporator and the concentrate was placed on a 10 cm Florisil column (2 cm i.d.) covered with 2 cm of sodium sulfate and eluted with 200 ml of n-hexane. The column had been initially washed with 100 ml of n-hexane. The eluate was concentrated to 5 ml in a Kuderna-Danish evaporator and then to 1 ml and 100 µl in a tube heater (Kontes, K720000). From the 1 ml concentrate a 10 µl portion was injected into the gas chromatograph. The purity of the solvents, materials and apparatus were checked by running blanks through the extraction + clean up procedure.

Electron Capture Gas Chromatography. A Hewlett-Packard Model 7620A gas chromatograph equipped with an electron capture detector (200 mCi tritium; pulse mode) was used. A silanized glass column (2 m x 2 mm i.d.) packed with 3 % OV-1 on 80/100 mesh Chromosorb W DMCS. Flow rates were: carrier gas helium, 40 ml/min, purge gas argon with 10 per cent methane, 60 ml/min. The temperatures were: injector 230°C, column 195°C, detector 215°C. The electron capture detector pulse interval was 50 µs.

Mass Fragmentography Instrumentation. A LKB 9000 gas chromatograph - mass spectrometer was used. The instrument was equipped with a mass marker and Multiple Ion Detector (MID, LKB item 9066). A 2 m glass column packed with 3 % OV-1 on 80/100 mesh Chromosorb W DMCS was used. The instrument was used under the following conditions: column 225°C; flash heater 250°C; separator 250°C; ion source 270°C; carrier gas, helium 20 ml/min; trap current 60 µA; gain 11; exit slit, 0.15 mm; entrance slit, 0.40 mm. Alternating between the three channels allows the continuous

monitoring of three different m/e on a UV-recorder with a paper rate of 2 cm/min.

Procedure. The reference substances of PCB containing four, five and six chlorine atoms were used for manual focusing on selected masses. The usual manner is to focus the magnetic field control on a reference ion introduced via the direct inlet probe. However, our experiences were that PCB introduced in this way still gave a high background after many hours. Therefore the reference PCB was injected in 10 ng portions into the coupled gas chromatograph and the magnetic field was manually refocused after roughly setting the mass marker. The ions containing one and two ^{37}Cl-atoms were brought into focus on the other two channels by lowering the accelerating voltage from ground (3.5 kV) while the magnetic field was held constant. This procedure was carried out manually during repeated injections and at the initial part of the eluting peak. The accuracy of the focusing was controlled during the elution of the compounds from the column. After the focusing procedure the different standards of PCB and river water extracts were run with the energy of the electron beam adjusted to 20eV. From the river water extracts 10 µl portions were injected into the coupled gas chromatograph; first from the 1 ml concentrate corresponding to 1.6 liters water; then from the 100 µl concentrate equivalent to 16 liters of water.

RESULTS AND DISCUSSION

Figure 1 shows the electron capture gas chromatogram of PCB standard Clophen A-60 (60% chlorine content) and the sample from river water. The sample gives a pattern which has simularities to the standard. However, at the start of the chromatogram there are differences particularly in the intensities of the peaks.

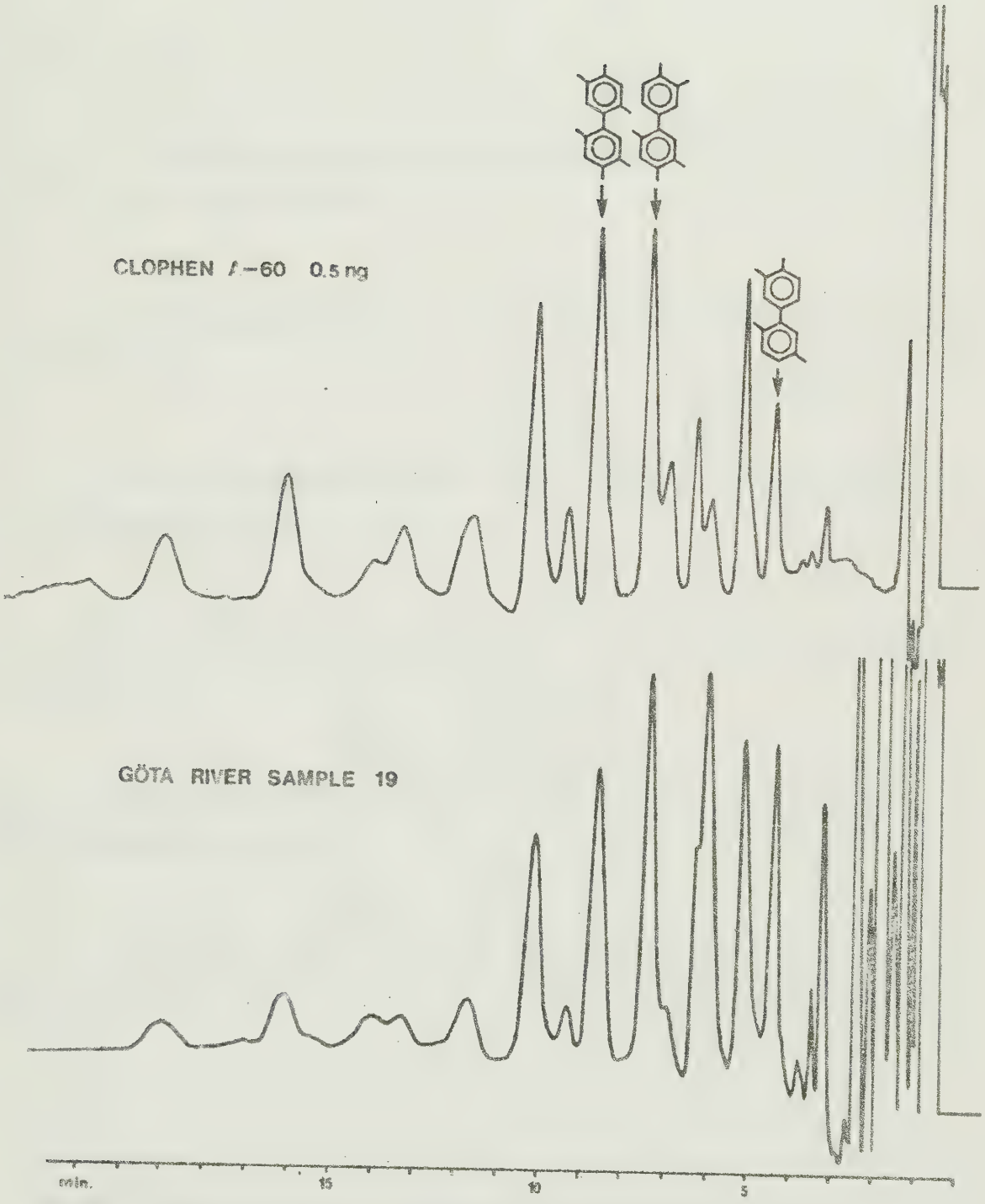

FIG 1

Gas chromatographic analysis with electron capture detector. The injected volume of river water extract correspond to 1.6 liters water.

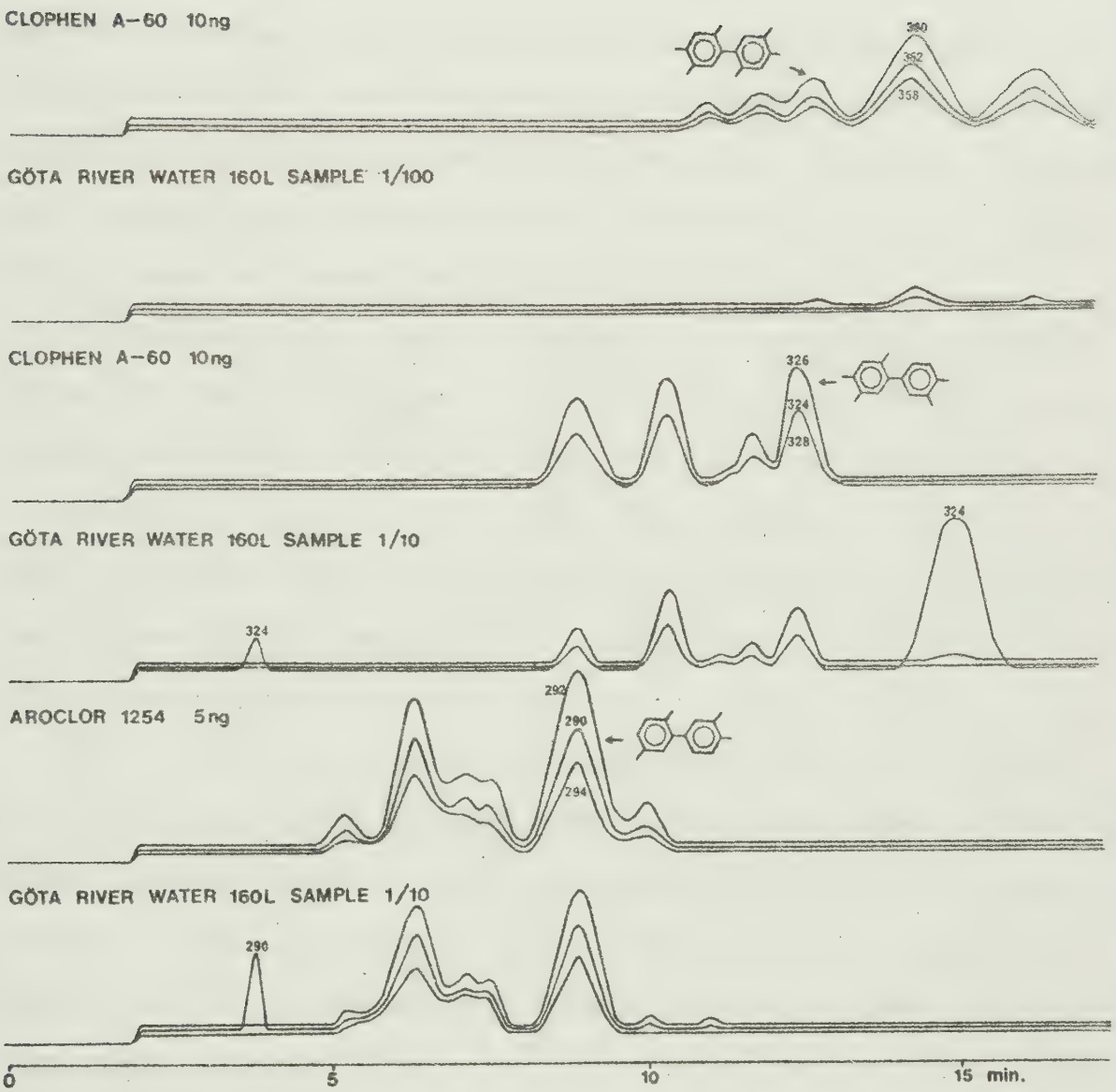

FIG 2

Mass fragmentograms of PCB standards and river water extracts.

This part resembles more the lower chlorinated biphenyls standard e.g. Aroclor 1254 (54% chlorine content).

Figure 2 shows the mass fragmentogram pattern from hexachlorobiphenyls, pentachlorobiphenyls and tetrachlorobiphenyls. The upper fragmentograms are derived from the different standards and the lower matching fragmentograms are from the sample. The sample run at the selected masses 358, 360 and 362 corresponding to hexachlorobiphenyls gave a weak response. Therefore the sample was further concentrated to 100 µl from which 10 µl was injected when focused on penta- and tetrachlorobiphenyls.

High specificity has been obtained by using the naturally occurring stable isotopes of chlorine. The ratio $^{35}Cl/^{37}Cl$ is roughly 1:0.32 and this will give the relative intensities of 1:1.28:0.61:0.13:0.01 for a tetrachlorobiphenyl ion with the m/e 290,292,294,296,298 of which the three first m/e were recorded. The characteristic multiplets of peaks have been used for confirmation as can be seen in Figure 2.

Three peaks from the river sample with m/e = 324 and 290 (fig. 2) are not PCB compounds since they lack signals from the heavy chlorine isotope. The ionization potential of 20eV was chosen to avoid fragmentation of higher chlorinated biphenyls giving rise to fragments with masses similar to lower chlorinated compounds. Safe and Hutzinger[14] have shown the successive expulsion of chlorine atoms and molecules at 70eV.

Quantitative measurements were not carried out with the mass fragmentography method, but with the electron capture detector. One reason is difficulties with certain instrumental parameters such as control of magnetic field intensity. These problems have recently beer solved by using an on line computer to control and automate the ion focusing.[15]

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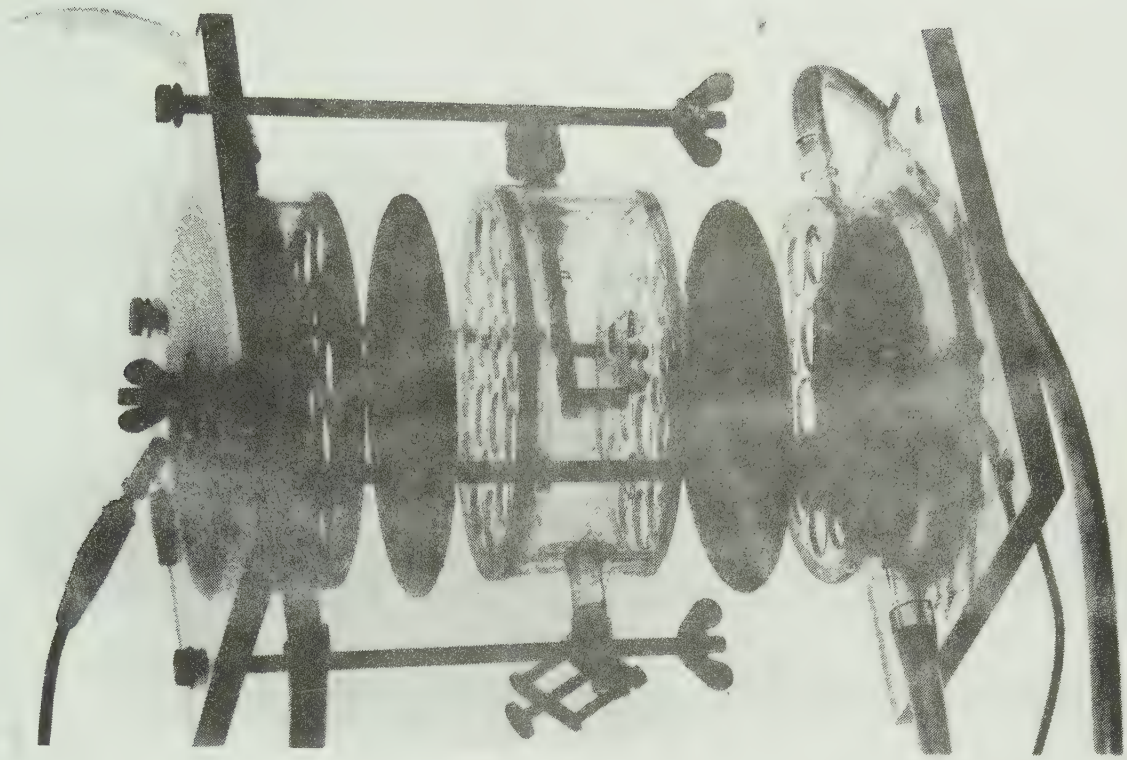
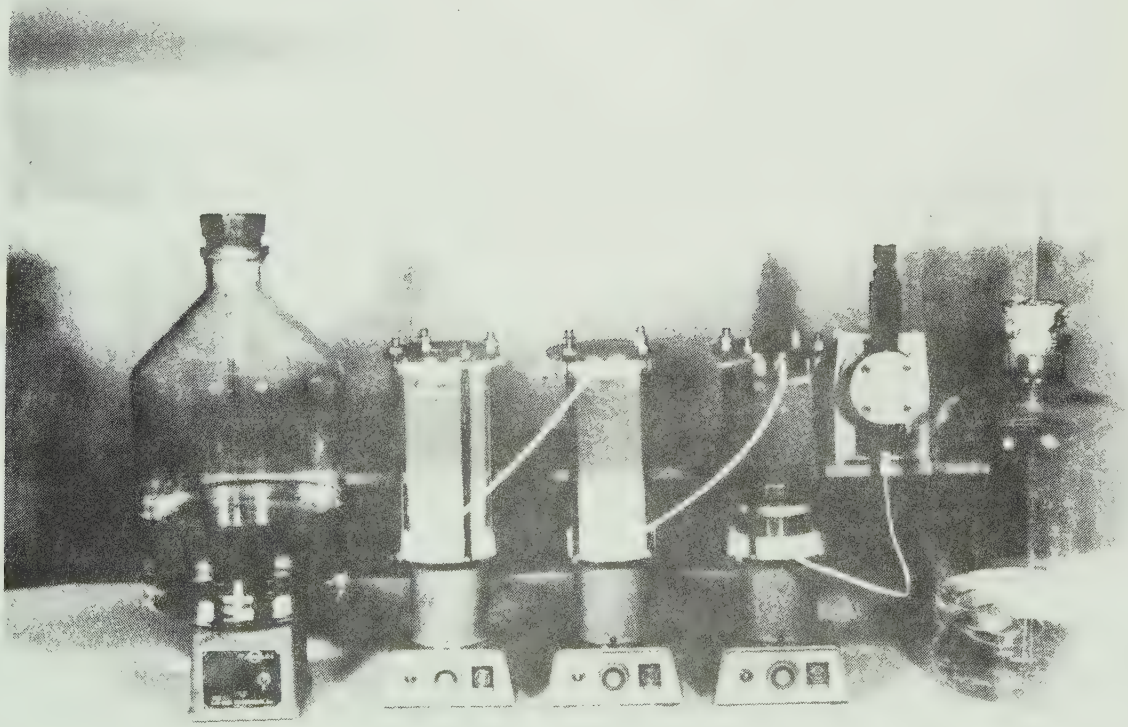


Electrodialys apparatus (430 ml volume).



A series extractor train (3 chambers).